Optimisation of Waste Stabilisation Ponds by Combining Duckweed-Based and Algal-Based Systems

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by

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EXECUTIVE SUMMARY

Waste water stabilization pond (WSP) technology is one of the most important natural methods for wastewater treatment, especially in rural areas. WSP systems are mainly shallow man-made basins comprising a single or several series of anaerobic, facultative or maturation ponds. The primary treatment takes place in the anaerobic pond, which is mainly designed for removing suspended solids, and some of the soluble element of organic matter (BOD). During the secondary stage in the facultative pond most of the remaining BOD is removed through natural biological and physical processes. The main function of the tertiary treatment in the maturation pond is the removal of pathogens and nutrients (especially nitrogen). Most WSP systems are algal based, but others depend on the growth of duckweed for nutrient removal. Waste stabilization pond technology is the most cost-effective wastewater treatment technology for the removal of pathogenic micro-organisms. The treatment is achieved through natural disinfection mechanisms. It is particularly well suited for tropical and subtropical countries because the intensity of the sunlight and temperature are key factors for the efficiency of the removal processes. Poor performance of WSP in developing countries can be attributed to both poor process design and poor physical design, as the systems have the potential to discharge high quality effluent compliant with the necessary standards.

Algal-based systems are dependent on a number of factors, of which available light is but one. Algae have a short doubling time. However, as cells multiply, the concentration increases, resulting in an increased turbidity. Less light penetrates and the growth of algae is limited. In this way, equilibrium is quickly established. Sufficient light is also required for bacterial destruction. Removal of faecal coliforms is effective in an algae-based WSP system. A disadvantage is that the algal cells remain in suspension and escape in the effluent. The presence of algae is indicated by a high COD and suspended solids concentration, often exceeding the general standards. This is one reason why WSP systems seldom comply.

Duckweed-based WSP systems have a distinctive floating mat of duckweed covering the surface of the pond. Systems such as these have recently been developed as a treatment option. It has been demonstrated that these systems are able to remove COD and nutrients effectively. Since they inhibit algal growth, the effluent is free from suspended material and therefore has a lower COD as compared with algae-

based WSP systems. The disadvantage is that production of oxygen is limited to the surface layer associated with the mat of duckweed, and the water column remains essentially anaerobic. Higher life-forms such as protozoa and their predators can therefore not be established. The important mechanism of grazing on bacteria is absent, thereby reducing the efficiency of faecal coliform removal. This explains why it appears that the ponds are under designed with respect to faecal coliform removal.

Based on the advantages and disadvantages of each treatment system, as well as observations made at existing pond systems, it was proposed that algal-based systems be combined with those of duckweed-based systems in an integrated manner, together with an aerated rock filtration step for effluent polishing before discharge of the effluent. It was hypothesized that the advantages of the algal system could mitigate the disadvantages of the duckweed system and vice versa, resulting in a final effluent of a better quality that what would be achievable with one system alone.

This study aimed to develop a conceptual process design for a combined system, based on laboratory scale experimental work. After conducting a thorough literature review it was found that while there was a wealth of information available on the design considerations for algal pond systems, there was a lack of information on duckweed-based systems, particularly with respect to the optimal growth conditions, expected nutrient uptake rates and recommended harvesting rates for removal of nutrients from the system. This study therefore focused on duckweed-based treatment.

Reactors were set up under various conditions of temperature, light intensity, nutrient concentration and harvesting frequency. They were mixed to avoid diffusion limiting conditions. Controlled temperatures tested included 13°C, 18°C, and 25°C, where reactors were set up in temperature controlled rooms under artificial light. Two reactors were set up under natural light conditions, one in the sun and one in full shade, where the temperature was not controlled. Different nutrient concentrations were supplied using dilutions of Huttner growth media of varying concentrations, and media was changed frequently to keep the concentration constant. Reactors were harvested to maintain culture ages of between 7d and 58d.

In addition to the reactors, smaller container tests were run at different temperatures, light intensities and harvesting rates in order to determine the nutrient uptake rates by the duckweed.

The following important observations were made from this study, which were considerations for the conceptual design:

- The surface density of duckweed in the duckweed ponds is important. If too high, the plants will have limited access to nutrients in the upper layers, and limited light, gas exchange and space to grow, reducing the potential for nutrient uptake. If the density is too low, however, algal growth will occur in the ponds due to poor attenuation of light.
- The harvesting rate is important, not only for the maintenance of the correct surface density, but also to allow for the generational capacity of the duckweed to reach its full potential. If the frequency of harvesting is too high, young plants will continually be removed from the system, which have the potential for exponential growth, and this could lead to the washout of plants from the system even at lower harvesting rates.
- At the concentrations of nutrients tested under the artificial light conditions with low light intensity, higher concentrations resulted in low growth rates and wash out of the cultures at the harvesting rates tested, especially at the lower temperatures of 13 and 18°C. This effect was not as severe in the reactors in the sun and shade at the same concentrations. It therefore appeared that light intensity and temperature are important for growth rate with higher growth rates and higher tolerance to high nutrient concentrations observed under high light intensities and warmer temperatures. For full scale duckweed systems this applies to the concentration of the water entering the duckweed pond. At lower temperatures it may be necessary to dilute the influent with either final effluent of the treatment system or of the duckweed ponds themselves through recycle. This may result in an increased surface area requirement.
- At lower nutrient concentrations, where duckweed were expected to be nutrient limited, it was observed under all temperatures and light intensities that the roots and fronds of the *Lemna* spp. increased in length and size in an effort to increase the surface area for absorption. This can be applied to full scale systems as an indication of plants under nutrient stress, which may indicate a need to increase the harvesting rate.

- Duckweed preferentially takes up ammonia nitrogen as a nitrogen source, rather than nitrate. Duckweed ponds must therefore precede algal ponds, rather than vice versa, as ammonia nitrogen will be converted to nitrate nitrogen through nitrification in the aerobic environment of algal ponds. It is also important that an anaerobic process precede the duckweed, where organic material can be mineralized and ammonia-nitrogen and ortho-phosphorus released in the bulk liquid.
- The light intensity and temperature applied to a mixed duckweed culture affected the species composition, with *Lemna turionifera* being the dominant species under high light intensity in the sun, and *Wolffia* spp dominating under medium light intensity in the shade.
- It is important that the duckweed layer not become diffusion limited, as this will result in low nutrient uptake. Introduction of turbulence in the duckweed treatment system is therefore a requirement, either by gentle mechanical mixing or through the use of baffles.
- Rock filters were initially suggested as a method for the removal of algal cells from the final effluent of the proposed combined duckweed-algal system in order to improve compliance with respect to COD and suspended solids in the effluent, as well as for the removal of any vestigial ammonia by aeration of the filters where necessary. In this study it become clear that the duckweed preferentially utilize ammonia as a nitrogen source, and there is therefore unlikely to be a high concentration of ammonia in the effluent of the duckweed ponds as it enters the algal ponds. Any ammonia remaining in the duckweed pond effluent will likely be nitrified by heterotrophic bacteria under the aerobic conditions of the algal ponds. It is therefore unlikely that the use of aerated rock filters for the nitrification of ammonia in the final effluent will be necessary. As an alternative to the rock filters that were initially proposed for the removal of algal cells from the final effluent, it is suggested that a final duckweed pond be implemented after the algal ponds and before discharge of the final effluent. Results of the current study indicate that duckweed are capable of survival at very low nutrient concentrations, and even continue to take up nutrients at these low concentrations. The shading effect of the duckweed will result in the death or senescence and sedimentation of the algal cells, resulting in a clear effluent. Although the nutrient concentrations will be low by this point, the duckweed will serve a secondary purpose of removing any remaining ammonia.

The results of the laboratory study were applied to develop potential conceptual designs for a pilot scale trial. Further research that was not possible on a laboratory scale level, for example the re-growth potential of algae following a duckweed treatment pond system, and large scale harvesting methods, is required to optimise the design for full scale application.

A draft operations and maintenance guide has been developed, as well as a training guide for full scale combined duckweed-algae stabilisation pond systems. These are based on the findings of the laboratory studies and the hypothetical application of the findings to full scale. These can serve as a basis for final documents, which will be prepared after the pilot phase of the project when the process design has been confirmed.

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CHAPTER 1 LITERATURE REVIEW

1.1 Duckweed

1.1.1 Taxonomy and plant structure

Duckweed belongs to the Lemnaceae family. The Lemnaceae comprise a distinctive group of diminutive, aquatic monocotyledons whose extreme reduction, miniaturization of organs, and cosmopolitan distribution contribute to their difficult taxonomy and systematics. The world's smallest angiosperms occur within this family, where some individuals may attain a width of only 0.3 mm at maturity. It is composed of small-sized monocotyledonous plants which float on the surface of stagnant or low water velocity pools, where water is rich in nutrients. The plant structure is relatively simple, devoid of distinct roots, stalks or leaves (Monette et al., 2006). The entire plant consists of a soft flat ovoid frond with a size of 2-20 mm (Alaerts et al., 1996). The worldwide spread of duckweed is due to its genetic adaptation leading to a wide variety of different species.

In 2002, Les et al. reported the results of a comprehensive phylogenetic analysis of Lemnaceae that was based upon the consideration of characters derived from molecular and non-molecular data and, in the former, from both nuclear and plastid genomes, using more than 4,700 characters that include data from morphology and anatomy, flavonoids, allozymes, and DNA sequences from chloroplast genes (*rbcL, matK*) and introns (*trnK, rpl16*). All data was reasonably congruent and contributed to strong nodal support in combined analyses. The combined data yielded a single, well-resolved, maximum parsimony tree with 30/36 nodes (83%) supported by bootstrap values that exceed 90%. Subfamily *Wolffioideae* was a monophyletic clade with 100% bootstrap support; however, subfamily *Lemnoideae* represented a paraphyletic clade comprising *Landoltia*, *Lemna*, and *Spirodela*. Combined data analysis confirmed the monophyly of *Landoltia*, *Lemna*, *Spirodela*, *Wolffia*, and *Wolffiella*. These analyses allowed them to formulate a relatively secure hypothesis of phylogenetic relationships within the Lemnaceae, which in turn served as the foundation for a revised, evolutionary classification of the family. The maximum parsimony cladogram of Lemnaceae species resulting from the combined analysis of morphological, flavonoid, allozyme and DNA sequence data is presented in Figure 1-1. About 40 species have been inventoried worldwide in various aquatic environments (Monette et al., 2006).

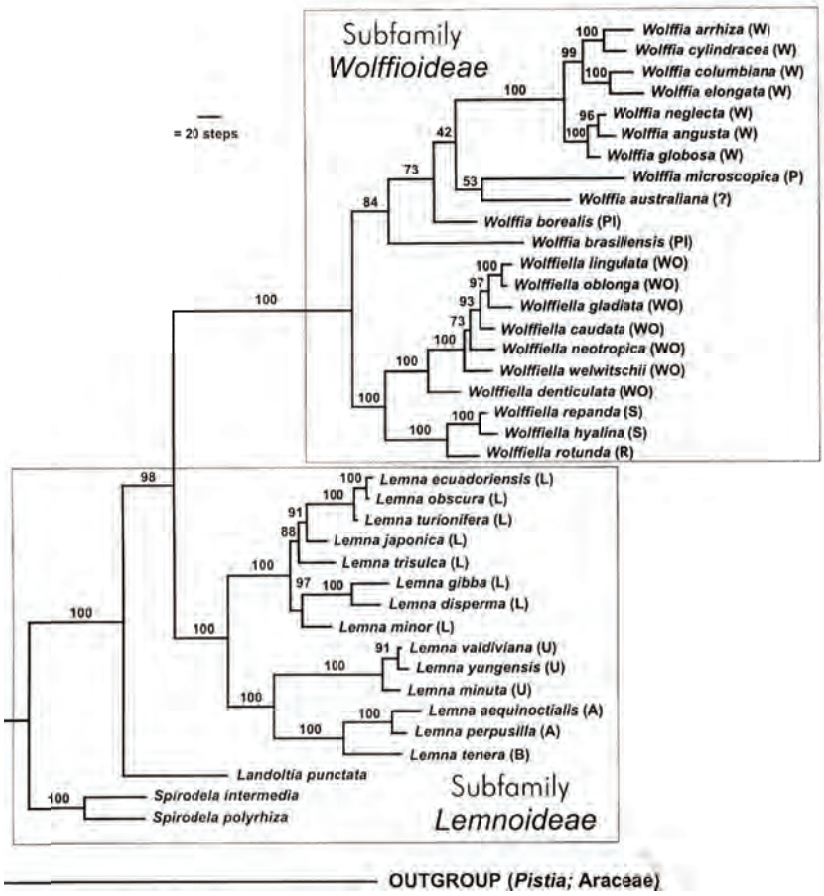

Figure 1-1: Maximum parsimony cladogram of Lemnaceae species resulting from combined analysis of morphological, flavonoid, allozyme and DNA sequence data. Shown is the single tree resulting from the analysis (2,674 steps; CI50.711; CI(exc.)50.643; RI50.875). Bootstrap support for nodes is indicated above branches. The two widely accepted subfamilies of Lemnaceae are indicated; subfamily *Wolffiodeae* is holophyletic; subfamily *Lemnoideae* is paraphyletic. Sectional designations are given in parentheses after each species. *Lemna*: A, *Alatae*; B, *Biformes*; L, *Lemna*; U, *Uninerves*. *Wolffia*: P, *Pseudorrhizae*; PI, *Pigmentatae*; W, *Wolffia*; ?, unassigned to section. *Wolffiella*: R, *Rotundae*; S, *Stipitatae*; WO, *Wolffiella* (Les et al., 2002).

1.1.2 Duckweed growth

1.1.2.1 Photosynthesis

Three modes of photosynthesis are known; C_3 plants, C_4 plants and CAM plants. In C_3 plants, the mesophyll cells contain well-formed chloroplasts and are arranged in parallel layers. The bundle sheath cells surrounding the veins do not contain chloroplasts. The Calvin cycle fixes carbon dioxide (CO_2) directly, and the first detectable molecule following fixation is PGA (3-phosphoglycerate), a C_3 molecule. In a C_4 leaf, the bundle sheath cells, as well as the mesophyll cells, contain chloroplasts, and the mesophyll cells are arranged concentrically around the bundle sheath cells. C_4 plants fix CO_2 to PEP (phosphoenolpyruvate, a C_3 molecule) forming a C_4 molecule prior to the involvement of the Calvin cycle. When the

weather is hot and dry, photorespiration occurs in C_3 plants but not in C_4 plants, giving C_4 plants an advantage under these conditions (Mader, 2001). Terrestrial C_3 and C_4 plants often may be distinguished from each other by their photosynthetic responses to increasing light and temperature; C_3 plants become photosynthetically saturated at one-third to one-half full sunlight while C_4 plants are not saturated even at full sunlight. Most C_3 plants also show an optimal temperature for photosynthesis near 20°C, while C_4 plants show decreasing photosynthesis only when the temperature exceeds 40°C. Aquatic macrophytes are not easily classified as C_3 or C_4 plants. Like C_4 plants, many aquatic plants have high light and temperature optima but like C_3 plants many aquatic plants show inhibition of photosynthesis with high oxygen levels. Wedge & Burris (1982) undertook a study to determine the light and temperature optima of *Lemna minor* L. and *Spirodela punctata* (G.F.W. Meyer) Thompson. Photosynthesis was measured both as oxygen evolution and $^{14}CO_2$, fixation. At temperatures ranging from 15 to 35°C, light saturation of photosynthetic O_2 evolution of *Lemna* occurred from 300-600 $\mu E.m^{-2}s^{-1}$, while in *Spirodela* photosynthetic O_2 evolution was light saturated at 600-1200 $\mu E.m^{-2}s^{-1}$. Photosynthetic O_2 evolution of both species was photo-inhibited at light intensities greater than 1200 $\mu E.m^{-2}s^{-1}$. The optimal temperature for *Lemna* photosynthetic O_2 evolution was 30°C, while the optimal temperatures for $^{14}CO_2$ fixation were from 20 to 30°C. For *Spirodela* maximum photosynthetic O_2 evolution occurred at 35°C, while maximum $^{14}CO_2$ fixation was at 30°C. The sensitivity of the young duckweed plants to photoinhibition by high light intensities (greater than 1200$pE.m^{-2}.s^{-1}$) may indicate though that they are closer to C_3 plants than C_4 plants, as C_4 plants are not photoinhibited at light intensities less than full sunlight. The responses of *Lemna* and *Spirodela* photosynthesis to temperature are not typical though of C_3 plants; temperatures above 20°C did not decrease photosynthesis, and therefore duckweeds instead seem to have the higher temperature optima characteristic of C_4 plants. Based on this experiment the authors concluded that duckweeds appeared to be C_3 plants, but that their high temperature optima and light intensities for saturation of photosynthesis make them atypical C_3 plants.

1.1.2.2 Effect of oxygen and carbon dioxide

Satake & Shimura (1983) measured carbon dioxide assimilation by the duckweed *S. polyrrhiza*, using a glass assimilation box and ^{14}C-$NaHCO_3$ under different water pH conditions. The authors found that *S. polyrrhiza* assimilated carbon dioxide from both the air and the water, and that assimilation from air was comparable to that from water under normal pH conditions. In 1986, Eshel & Beer again studied the relative importance of inorganic carbon assimilation from the gas versus aqueous phase by the floating duckweed *Spirodela polyrrhiza*, but found that carbon assimilation from the aqueous phase amounted only up to 5% of that from the air, and that no direct effect of pH on this process was detected. They therefore disputed the results of Satake & Shimura (1983), based on the

influence of the rate of gaseous exchange of $^{14}CO_2$ between the solution and the gas phase, and the apparent failure of the authors to properly recognize the effect of pH on inorganic C concentrations and on ^{14}C specific activity.

Dry weight and relative growth rate of *Lemna gibba* were significantly increased by CO_2 enrichment up to 6000 µl $CO_2.l^{-1}$, in a study performed by Anderson et al. (1985). This high CO_2 optimum for growth was probably due to the presence of non-functional stomata. The response to high CO_2 was less or absent following four days growth in 2% O_2. The leaf area ratio decreased in response to CO_2 enrichment as a result of an increase in dry weight per frond. The rate of photosynthesis was increased by CO_2 enrichment up to 1500 µl CO2.l^{-1} during measurement; showing only small increases with further CO_2 enrichment up to 5000 µl $CO_2.l^{-1}$ at a photon flux density of 210 $\mu mol.m^{-2}.s^{-1}$ and small decreases at 2000 $\mu mol.m^{-2}.s^{-1}$. The actual rate of photosynthesis of those plants cultivated at high CO_2 levels, however, was less than the air grown plants. The response of photosynthesis to O_2 indicated that the enhancement of growth and photosynthesis by CO_2 enrichment was a result of decreased photorespiration. Plants cultivated in low O_2 produced abnormal morphological features and after a short time showed a reduction in growth.

Increasing atmospheric concentrations of carbon dioxide (CO_2) and ozone (O_3) prompted Bailey et al. (1999) to evaluate the combined effects of these gases on duckweed growth and physiology. The primary goal of the study was to investigate the response of two species of duckweed, *Lemna minor* L. and *Spirodelapolyrhiza* (L.) Schleiden, to projected future ambient levels of O_3 and CO_2 under realistic field conditions as measured by growth and gas exchange. The two duckweed species were treated with either charcoal-filtered air (CF), ambient O_3 ($1XO_3$), twice ambient O_3 ($2XO_3$), twice ambient CO_2 plus twice ambient O_3 ($2XCO_2+2XO_3$), or chamberless open-air (OA). Two experiments were conducted. In Experiment I, *L. minor* was treated for 15d with a cumulative O_3 exposure of $14.4ppm.h^{-1}$. No O_3 effects were observed during Experiment 1. Dry weight of individual fronds and photosynthesis per frond increased in Z. *minor* exposed to $2XCO_2+2XO_3$- air. In Experiment II after 25 d of treatment (cumulative O_3 exposure of $16.2ppm.h^{-1}$), negative effects of $2XO_3$ on the photosynthetic and growth rates of *L. minor* were observed. Dark respiration of *L*. *minor* significantly increased in $2XO_3$-air compared with controls, but declined significantly in $2XCO_2+2XO_3$-air compared to those grown in $2XO_3$-air. Photosynthesis and dry weight per frond increased in $2XCO_2+2XO_3$-air when compared with all other treatments. Measurement of A/C. (assimilation versus intercellular CO_2 concentration) curves in *L*. *minor* showed a significant reduction in carboxylation efficiency and maximum rates of photosynthesis in $2XCO_2+2XO_3$-air compared with other treatments when expressed per weight. No differences in carboxylation efficiency were detected between treatments when expressed

per frond. After 25d of treatment, photosynthesis (per frond) and dry weight of *S. polyrhiza* were reduced in $2XO_3$-air, but final frond number was unaffected. Dark respiration of *S. polyrhiza* was unaffected in $2XO_3$- air, but when exposed to $2XCO_2$+$2XO_3$-air, it declined significantly. Although *S. polyrhiza* photosynthesis per frond increased in $2XCO_2$+$2XO_3$-air, dry weight was unaffected when compared with all other treatments. Only when comparisons were made between *S. polyrhiza* grown in $2XCO_2$+$2XO_3$ -air and $2XO_3$-air, were significant increases in dry weight observed. The addition of $2XCO_2$ to $2XO_3$-air resulted in amelioration of negative O_3 effects for most responses for both duckweed species.

1.1.3 Application of duckweed in water treatment

Domestic wastewater treatment is of urgent concern in developing countries because of population pressure and urbanisation. Algae-based lagooning can be a cost-effective treatment method provided land is available and cheap. However, operational problems regularly occur, and the effluent usually contains large amounts of algal matter, which upon decomposition generates BOD and releases nutrients in the receiving water. It has been suggested that sewage lagoons with floating macrophytes also purify sewage and could generate valuable biomass. Water hyacinth, for example, growing profusely on sewage, promotes good effluent quality (Alaerts et al., 1996).

In comparison with other macrophyte-based systems, for example those using the floating water hyacinth *Eichhornia crassipes* L. Solms, or rooted emergent heliophytes, sewage treatment systems involving duckweed appear to have an advantage for application in nutrient and pollutant reduction. This is mostly due to their higher relative growth rates, which can be as high as 0.3 natural log units.day^{-1}, compared to other herbaceous angiosperms. This is coupled with their small size, short life spans, high nutrient requirements (Vermaat & Hanif, 1998), a low fibre and high protein content of 30-49% dry weight (Caicedo et al., 2000; Oron, 1994) and relative ease of harvesting. Treatment efficiency of duckweed-based systems for biological and chemical oxygen demand (BOD and COD) is similar to that of conventional stabilisation ponds, but removal of suspended solids is usually better in duckweed-based systems, due to suppression of algae growth (Van der Steen et al., 1999). Another advantage of duckweed-based systems is that nutrients can be partly recovered rather than lost to the atmosphere, or removed with the effluent (Caicedo et al., 2000). Oron (1994) reported that the annual yield (dry matter) of duckweed, harvested two to three times a week, is 55 ton/ha The economic benefit of the additional by-product of the biomass reduces wastewater expenditures in the range of 0.020 to US$0.050 per each treated m^3 of wastewater.

Bacterial decomposition causes anaerobiosis, which is maintained by the duckweed mat that prevents aeration. The mineralized nutrients are then the main source for duckweed growth. Duckweed species such as *Spirodela* and *Lemna* reduce the oxygen content of the wastewater; however, this anaerobiosis does not seem to cause any damage to the plants or prevent the effluent from being re-used for irrigation. The main inorganic compounds which are converted into protein by the duckweed vascular plants are HCO_2^- or CO_3^{2-}, NH_4^+ and PO_4^{3-}. In a system consisting of one reactor only (single stage), the CO_2 produced by the bacteria provided an enriched environment for plant growth (Oron et al., 1988). For these reasons, duckweed has been used more and more frequently in the treatment of household and agricultural wastewater, especially in the last two decades. They have been shown to be excellent fodder for fish, poultry and cattle, and the harvested material duckweed can therefore create a substantial financial incentive for controlled faeces and wastewater collection and treatment in both rural and urban areas (Alaerts et al., 1996).

A series of biotic and abiotic factors, such as temperature, growth medium composition, light intensity and mat density, exert significant influence on duckweed growth. It appears that there exist optimal values of temperature, pH, composition of the nourishing medium and light intensity beyond which the plant growth is slowed down and even stopped (Monette et al., 2006).

1.1.3.1 Nutrient requirements and adsorption/removal

1.1.3.1.1 Effect of pH and nitrogen compounds on duck weed growth

The nitrogen in anaerobic effluent is present mainly as ammonium (NH_4^+). This is an advantage because duckweed has a preferential uptake of ammonium over other sources of nitrogen (Oron et al., 1988). However, the ammonium ions are inhibitory to duckweed growth at high concentrations. The inhibition by total ammonia ($NH_4^+ + NH_3$) has commonly been attributed more to the NH_3 form than to the NH_4^+ form. The pH of the growth medium or wastewater determines the ratio between the two species and therefore the NH_3 concentration. The un-dissociated and uncharged NH_3 molecule is lipid-soluble and therefore easily enters plant cells through their membrane and disturbs the cell metabolism. Biological membranes are relatively impermeable to the ionised and hydrated form, NH_4^+, which is generally thought to be less detrimental for duckweed growth, although it has been suggested that high NH_4^+ concentrations result in strong depolarisation of the membrane. This could result in a general inhibition of anion transport. Caicedo et al. (2000) performed laboratory scale renewal fed batch experiments to assess the effect of total ammonia ($NH_3 + NH_4^+$) nitrogen and pH on the growth rate of the duckweed *Spirodela polyrrhiza*. The experiments were performed at different total ammonia nitrogen concentrations, different

pH ranges and in three different growth media. The relative growth rate of *Spirodela polyrrhiza* under the experimental conditions was found to decrease with increasing concentrations of total ammonia. The inhibition of duckweed growth by ammonium was found to be due to a combined effect of ammonium ions (NH_4^+) and ammonia (NH_3), the importance of each one depending on the pH. It was concluded that if growth inhibition should be controlled at, or below, 30% then total ammonia concentrations in the duckweed pond should be below 50 mg/l, while pH should be maintained below 8 whereas for ammonium concentrations between 50 and 100 mg/l N, the pH should not be higher than 7.

Körner et al. (2001) conducted similar laboratory scale batch experiments under controlled conditions at different total ammonia concentrations (10-300 $mgN.l^{-1}$) and controlled pH values of 6.8-8.7 using settled domestic wastewater to measure the effect of the ionised ($NH4^+$ or ammonium) and un-ionised form (NH_3) on the growth of the duckweed *Lemna gibba*. Relative growth rates (RGR) varied between 0 and 0.3 per day. The toxicity of total ammonia to duckweed was a result of the effect of both, ionized and un-ionised, forms at low NH_3 concentrations (<1 $mg.N.l^{-1}$). At higher NH_3 concentrations, the toxic effect of the ionised form could be disregarded. Relative growth rates of *L. gibba* decreased linearly with increasing NH_3 concentrations up to a maximum level (8 $mgN.l^{-1}$), above which duckweed died. This data indicated that *L. gibba* can be used to treat wastewater containing high total ammonia concentrations as long as certain pH levels are not exceeded, as was concluded by Caicedo (2000) for the duckweed *Spirodela polyrrhiza*. Extrapolated relative growth rates resulting from different combinations of pH and total ammonia were calculated given for the examined ranges. Up to a pH of 7.8, a substantial production of 55 $kgDW.ha^{-1}$ per day was achieved. Wastewater treatment using *L. gibba* becomes impossible at pH levels above approximately 9.8, depending on the temperature.

1.1.3.1.2 Nitrogen and phosphorus removal

Duckweed displays fast reproduction through gemmation (budding) and can absorb large amounts of nutrients such as nitrogen (N) and phosphorus (P). These nutrients are removed through the harvesting of the duckweed from treatment systems. In 1988, Oron et al. evaluated the nitrogen removal and conversion by duckweed grown on waste water. Growth of the plant was examined at two pond depths and various hydraulic retention times. Operating the ponds under a retention time of up to 5 days and depth ranging between 0.20 and 0.30 m was found to be advantageous. The preferable operational regime was also verified by ammonia removal. The increase in ammonia removal rate was the steepest in the range of 3-5 days. A deflection in the removal function was identified at a retention time of about 5 days and the related removal obtained was about 80%. Removal efficiency was over 90% only at an extended retention time of 10 days. This indicates that the quantity of

nitrogen oxidized or assimilated depended mainly on the organic load. Dry yield of the duckweed approached 15 $g.m^{-2}day^{-1}$ with a protein content of about 30% in the short retention-time treatments. The pH conditions (which were maintained at 7) and the organic load, guaranteed adequate conversion of ammonia into protein. Prevention of failure was due to lack of inorganic carbon under low pH or free ammonia generation under high pH. An increase in retention time was associated with a decrease in growth rate, reduction in yield and protein content. Temperature was found to affect the biological process in two ways; oxygen and ammonia transfer in the fluid and the rate of biodegradation of the organic matter. Temperature induced stratification was indeed detected in the ponds and influenced the efficiency of the whole treatment process.

In order to assess the performance of a full scale duckweed-covered sewage lagoon which had been operated successfully for more than four years, Alaerts et al. (1996) conducted detailed sampling during the dry season, when the hydraulic retention time was 20.4d. The surface loading rate was 48-60 kg BOD/ha.d. Concentration reduction was 90-97% for COD, 95-99% for BOD, and 74-77% for Kjeldahl-N and total P. 42-47% of the N and P load was removed by the Lemnaceae; 27-32% disappeared through percolation and side seepage and 7-13% through sedimentation. The final effluent contained 2.7 mg Kjeldahl-N/l and 0.4 mg total-P/l. The water column remained aerobic. At two-thirds of retention time the plants had absorbed virtually all NH_4^+ and ortho-PO_4^{3-} from the water column. There was a one-fifth loss of inflow volume as a result of seepage during the dry season. However, the authors estimated that the duckweed harvest would remove 60-80% of the N and P load in a water tight lagoon, or 0.26 g $N/m^2.d$ and 0.05 g $P/m^2.d$ in the first three-quarters of retention time. Corrected for the leakage, plant productivity under these fertilised and managed conditions was sustained for several years at the level of 58-105 kg(dw)/ha.d, or 715-1200 kg/ha.d (over full lagoon surface) in the dry and wet season, respectively. The authors suggested that the microbial hydrolysis of the more complex organic N and P into NH_4^+ and ortho-PO_4^{3-} was the limiting step for enhanced biomass production, as the intensive harvesting (every 2-3d) reduced the possibility that nitrifying bacteria (or cyanobacteria fixing atmospheric nitrogen) would thrive on the plants' surface or root zone. It was therefore concluded that it is important to provide adequate pre-treatment for the sewage to release organically bound NH_4^+ and ortho-PO_4^{3-} (e.g. anaerobic up-flow sludge blanket treatment).

Many studies feature incomplete mass balances that fail to distinguish between duckweed and its attached periphyton, and do not address losses due to sedimentation, denitrification, or ammonia volatilization and therefore often have proportions of the mass balance unaccounted for. Vermaat & Hanif (1998) assessed the performance of five duckweed species (*Lemna gibba* L., *Lemna minor* L., *Lemna trisulca*, L., *Spirodela polyrhiza* (L.) Schleiden

and *Wolffia arrhiza* (L.) Hork. ex Wimm.) in laboratory scale experiments comparing domestic sewage and two types of artificial waste water with a standard mineral growth medium. In a subsequent 12d batch experiment with *Lemna gibba* in settled domestic waste water, a detailed mass balance was established for nitrogen (N) and phosphorus (P). All species yielded less on the artificial waste water than in the mineral growth medium, however *Spirodela polyrhiza* and *Lemna gibba* performed equally well in domestic waste water when compared with the mineral growth medium. In the batch experiment, 77% of the total-P was removed, of which 18% was attributed to associated periphyton. There was a 94% reduction in Kjeldahl-N, however 50% of the removal was attributed to denitrification as the pH was too low (<8) for NH_4 volatilisation. This lead to the conclusion that the denitrifying bacteria associated with duckweed play an important role in N removal in duckweed systems.

These studies by Alaerts et al. (1996) and Vermaat & Hanif (1998) report incomplete mass balance equations and the authors concluded that ammonia volatilisation accounts for only a small percentage of overall nitrogen removal. Since the generally neutral pH of duckweed-based ponds does not support ammonia volatilisation, it was assumed that the nitrogen that could not be accounted for in these studies was due to nitrification and subsequent denitrification. Zimmo et al. (2000) found a nitrogen loss of 32% and 13% in algae and duckweed batch experiments, respectively, that was attributed to ammonia volatilisation and denitrification.

Al-Nozaily et al. (2000) assessed the effect of depth, mixing and nutrient concentration on duckweed growth and the uptake of nutrients by duckweed in batch reactors under conditions that reflect those in the field. Potential nitrification was excluded by the addition of a nitrification inhibitor. The contribution of duckweed (*L. gibba*) to N and P removal was studied at NH_4^+ concentrations of 25-96 mg/l in 10, 30, 70 and 95 cm deep reactors, and liquid mixing intensity of 0, 0.3, 1.0, 2.3 and 34.1 W/m^3. The duration of each experiment was 20d with biomass harvesting every 5d. For a given N input, depth as an independent variable did not affect overall N removal except through increase of surface loading, whereas mixing had a significant positive effect at high Kjeldahl nitrogen concentrations. TP removal however, was proportional to depth rather than to the initial loading concentration. At a low nitrogen surface loading concentration of 183 kg N/ha, TN removal (uptake plus losses) could be completely attributed to duckweed uptake, whereas at a high loading concentration (>300 kg N/ha), which correlated with a high NH_4^+ concentration, N uptake less than 50% of the total removal. The achieved TN and TP removal rates ranged from 2-10 kg $N/ha.d^{-1}$ and 0.4-1.1 kg $P/ha.d^{-1}$, respectively, depending on the initial loading concentration, with 1-4.8 kg $N/ha.d^{-1}$ and 0.13-0.58 kg $P/ha.d^{-1}$ removed by duckweed

uptake. Mixing significantly enhanced the TP removal rate, as well as the duckweed uptake, indicating that the NH_4^+ inhibition was alleviated. Relative growth rate decreased from 0.19 to 0.05 d^{-1} for initial concentrations of 25-96 mg NH_4^+ -N/l, respectively. N and P removal followed first-order kinetics.

In 2003, Zimmo et al. assessed the relative contribution of ammonia volatilisation in overall N-removal from domestic waste stabilization ponds. Ammonia volatilization rates were measured in pilot plant facilities consisting of one line of four algae-based ponds in series and a parallel line of four ponds with a floating mat of duckweed (*Lemna gibba*). Ammonia volatilisation was assessed during a period of one and a half years. The ammonia volatilisation rates in algae-based ponds were higher than in duckweed-based ponds. Ammonia volatilisation was highly correlated with NH_3 concentration in pond water, which in turn was governed by the combined effect of pH and water temperature. Shading provided by the duckweed cover prevented strong diurnal changes in pond water pH such as commonly observed in conventional stabilisation ponds. The lower pH in duckweed-based ponds in comparison with algal-based ponds resulted in lower ammonia concentrations and hence in lower ammonia volatilisation. The duckweed cover appeared not to provide a physical barrier for volatilisation of un-ionised ammonia, because whenever NH_3 concentrations were equal in both sets of ponds, the volatilisation rates were also equal. Volatilisation was in the range of 7.2-37.4 mg-N m^{-2} d^{-1} and 6.4-31.5 mg-Nm^{-2} d^{-1} in the algae- and duckweed-based ponds respectively. Volatilisation rates decreased along the series of ponds as the un-ionised ammonia concentration decreased. Average influent and effluent ammonium nitrogen measurements showed that the ammonia volatilisation during the study period in either system did not exceed 1.5% of total ammonium nitrogen removal. Therefore this study confirmed results from their previous laboratory study indicating that nitrification/denitrification, rather than ammonia volatilisation, is the most important mechanism for N removal in both algae-based and duckweed-based ponds.

In 2004, Zimmo et al. took their research further by compiling nitrogen balances incorporating the main nitrogen fluxes in algal-based and duckweed-based pond systems under three different operating conditions. Nitrogen removal in duckweed ponds was lower by 20%, 12% and 8% compared to removal in algal ponds during cold temperature, warm temperature and periods of high organic loading, respectively. The important nitrogen fluxes in algal systems were sedimentation and denitrification whereas harvesting of duckweed, denitrification and sedimentation were the major nitrogen fluxes in duckweed systems. Ammonia volatilization in both systems did not exceed 1.1% of influent total nitrogen in the treatment systems. Algal-based ponds showed higher nitrogen removal via sedimentation (46-245% higher) compared to duckweed-based ponds. The difference increased with higher

temperature and lower organic loading rates. Algal systems also showed higher nitrogen removal via denitrification (7-37% higher) and ammonia volatilisation (7-51% higher) compared to duckweed systems. N-uptake by duckweed corresponds to 30% of the influent nitrogen during warm/low organic loading period and decreased to 10% and 19% during the cold and warm/high organic loading period, respectively. Predictive models for nitrogen removal presented a good reflection of nitrogen fluxes on overall nitrogen balance under the prevailing experimental conditions. Validation of the models for algal-based pond systems and duckweed-based pond systems with reported data from literature gave poor results for shallower ponds, while better agreement was obtained using data for deeper ponds. It was concluded that surface area per pond volume plays an important role in nitrogen removal from pond systems.

N transformation processes are relatively complex in a duckweed wastewater treatment pond. Nitrogen not only exists in different media, such as algae, duckweed, sediment and suspended organic particles, but also presents in various N forms, such as NH_3-N, NO_2-N, NO_3-N, and organic N ($N_{org)}$. Furthermore, some parameters, such as DO, water temperature, pH, and light intensity, also affect these transformations. Therefore with the annual periodic variations of environmental factors, N transformation processes change significantly. Routine monitoring cannot quantify the transformation fluxes of different N forms, thus cannot effectively predict the operational performances of a duckweed pond. Most of the proposed models for N removal, including those described above, concentrated on the transformations of total ammoniacal nitrogen (N_{am}), N_{org} and nitrate/nitrite (N_{ox}) in traditional primary facultative ponds, and the contributions of hydrophytes were not included in these models, though they played important roles in N cycling With this complexity in mind, Peng et al. (2007) saw the necessity to develop a simple and effective N transformation model for the optimization of the duckweed pond design and operation to effectively describe the dynamic processes of different N forms in water bulk, sediment, and hydrophytes of an operational duckweed pond. Based on the dynamics of N_{am}, N_{ox} and organic nitrogen contained in sediment ($N_{sed)}$, in water ($N_{org)}$, and in hydrophyte pools (N_{algae} $+N_{dw}$), respectively, and also including the influence of various environmental factors (i.e. pH, DO, T and hydraulic retention time) a mathematical model was developed to predict N transformation processes in duckweed ponds. The principal N-compounds, N-transformations and N-fluxes in the model are conceptualized in Figure 1-2. In this model, N_{am} may be transformed via a number of pathways: converted to nitrite/nitrate via nitrification processes, assimilated by hydrophytes (mainly in forms of algae or duckweed), volatilized as gaseous ammonia, or discharged during water exchanges. In this model, both hydrophyte assimilation and denitrification (taking into account that denitrification could occur on the surface of sediment through the water exchange between water bulk and

sediment) were included to improve the precision of the predicted values. The decomposition of algae was viewed as the major source of N_{org} because the decomposition mostly occurred in water column. However, the decaying duckweed usually settled directly on the surface of sediment, and was then decomposed. Therefore, the N_{dw} was assumed as a source of N_{sed} (N contained in sediment), and the conversion from N_{dw} to N_{org} was excluded in the simulation. N transformation in sediment was simplified, because N mainly existed in the form of organic nitrogen in sediment, and the contents of N_{am} and N_{ox} were normally less than 0.5% of the TN. Moreover, due to relatively low exchange fluxes between water and sediment, the adsorption/release of N_{am} and N_{ox} could be ignored to a large extent. Therefore, only the N_{sed} re-mineralization, N_{org} precipitation/ adsorption, and N_{sed} mortality were included in N_{sed} transformation pathways.

The availability and the sensibility of the transformation model were investigated correspondingly. The results showed that the transformation model could exactly simulate the annual variations of N_{am}, N_{ox}, N_{org}, N_{algae}, N_{sed} and N_{dw} with correlation coefficients of 0.89, 0.72, 0.61, 0.74, 0.86 and 0.71, respectively. The simulated values of N_{org} were much lower than the measured values in windy winter, resulting in the lower correlation coefficient between them. However, the simulated values of N_{org} were much lower than the measured values in windy winter, resulting in the correlation coefficient between them only being 0.61. Sensitivity analysis revealed that the model was a stable simulation system except the variable of N_{dw}, and the authors concluded that further research should concentrate on a better understanding of the N transformation in duckweed.

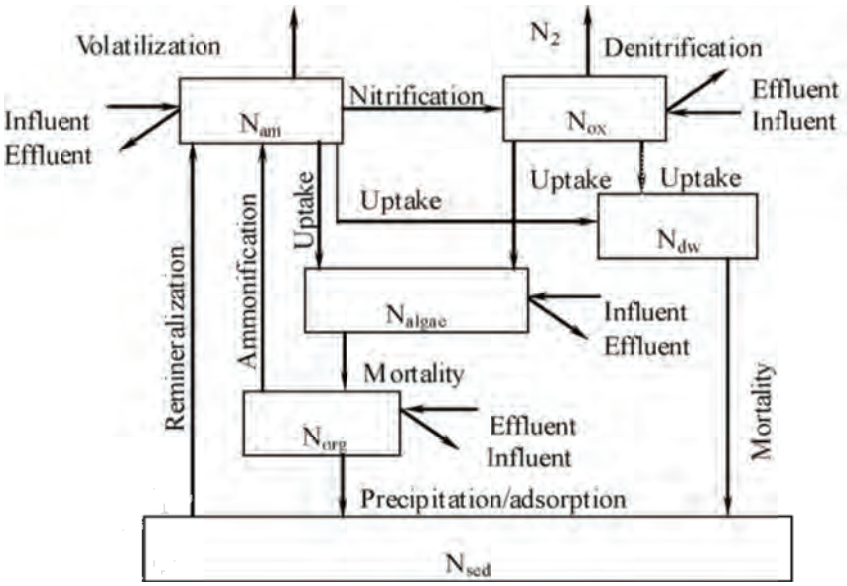

Figure 1-2: The principle N-compounds, transformations and fluxes in the model developed by Peng et al. (2007)

In a pilot study conducted by El-Shafai et al. (2007), an up-flow anaerobic sludge blanket (UASB) reactor was followed by three duckweed ponds containing *Lemna gibba*. During the warm season, residual values of ammonia, TKN and total phosphorus were 0.41 mg N/l, 4.4 mg N/l and 1.11 mg P/l, with removal efficiencies of 98%, 85% and 78%, respectively. The N removal consisted of 80% by plant uptake and conversion to protein on average 4.42 kgN/ha.d., 5% by sedimentation and 15% was unaccounted for, which was assumed to be due to removal by denitrification. The total P recovered by duckweed ranged from 0.97 kg P/ha.d in the first duckweed pond, 0.94 kg P/ha.d in the second pond and 0.86 kgN/ha.d in the third pond. In winter, the TN recovered by the plants was 1.21, 1.46 and 1.28 kg N/ha.d in the three duckweed ponds respectively, and the TP recovery values were 0.27, 0.32 and 0.29 kg P/ha.d respectively. The nutrient recovery from the UASB effluent in duckweed ponds was therefore found to be duckweed growth rate dependent, and because the growth rate of duckweed was significantly reduced by the lower temperature in winter (13-20°C), the nutrient recovery decreased in winter.

1.1.3.2 Effect of duckweed decomposition

If the duckweed cover is too thick, the lower plants of the duckweed cover may not be exposed to light. When the plants are not harvested they become senescent rapidly and finally decompose. Through the decomposition a large amount of nutrients (nitrogen, phosphorus) and organic material is released from the dying plants into the water. Consequently the purification efficiency of the pond system decreases, and the quality of the effluent becomes worse. Szabó et al. (2000) investigated the elemental dynamics of decomposition of *Lemna gibba* in the presence and absence of wastewater micro-organisms, to determine the impact of microbial degradation and leaching on duckweed decomposition over a 200 day period under laboratory conditions. The residual mass of plant litter in the decomposition vessels decreased three times more rapidly under biotic than abiotic conditions. The organic matter in the duckweed litter lost about half its weight within 67.9 days in the presence of micro-organisms while more than 200 days were required in axenic vessels. In the former case, ash free dry weight (AFDW) loss followed an exponential pattern of decay. The rate constant was $0.0102.day^{-1}$ and the decay was virtually complete after 200 days. The C and K concentration of the remaining duckweed litter decreased; the N, Ca, Fe and B concentration increased in both treatments. The concentration of total N, P, K, Mg, and Mo increased in the receiving water in both treatments but was much higher under biotic than abiotic conditions. Mass balances of nutrients in the vessels and flux of these nutrients between compartments in the vessels (duckweed litter, water and sediment) were determined. Under axenic conditions the release of elements was very slow, with only a notable leached quantity of potassium observed. Leaching of potassium, magnesium and organic carbon took place mainly during the first term of incubation and then slowed down.

Under biotic decomposition the elemental content of the litter decreased by more than 50% over 43 days for K, 53 days for Mo, 64 days for C, 81 days for Mg, 101 days for S, 104 days for P, 108 days for Na, 111 days for N, 140 days for B. Calcium and iron were immobilised in the litter. Most of the released N, S, P, K, Mg and Mo remained in the water, but B and Mn settled into the sediment. The result of the investigation demonstrated that the nutrient flux from decomposing duckweed litter is mainly a microbially mediated process. It is therefore important that duckweed be harvested regularly to prevent decomposition and return of nutrients to the water.

1.1.3.3 Oxygen balance and COD/BOD removal

Duckweed pond systems remove organic matter primarily through aerobic heterotrophic oxidation. For this, the active diffusion or transportation of oxygen into the liquid phase is required. It has been suggested that aquatic weeds act as a ``biofilter'' by providing attachment opportunities for aerobic heterotrophic bacteria. Dissolved oxygen (DO) transfer is influenced by reactor depth, time of the day, and the degree of wind-induced turbulence of the water surface. In duckweed lagoons (0.5-1.5 m) re-aeration through the surface may be obstructed by the duckweed mat. Alaerts et al. (1996) reported that a full scale duckweed pond system had a fairly constant high DO of 2-4 mg O_2/l along the whole length of the pond, which suggested adequate re-aeration, which in this case might have been caused by the low BOD concentration of approximately 100 mg/l at the inlet. The optimal depth of a duckweed sewage pond should be related to the ratio of the oxygen consuming wastewater volume to the duckweed-covered surface area. The latter determines the O_2 flux into the wastewater, and may thus enhance COD removal in the water column. The vertical transport fluxes of oxygen and nutrients in the water column and the volume-to-surface area ratio determine the maximal depth that can be applied.

Laboratory scale experiments on duckweed-covered domestic sewage were carried out by Körner et al. (1998) to determine whether removal of organic material was faster in the presence of duckweed, and to determine the role that duckweed play in the degradation of organic material. Performance of systems containing axenic and non-axenic *Lemna gibba* L., artificial plastic duckweed, air bubbling pumps and a combination of the latter two were compared with a control system without duckweed. Removal of COD was significantly faster in the presence of duckweed. Removal efficiencies after 3d were 74-78% in duck-weed-covered treatments compared to 52-60% in uncovered controls. DOC levels remained constant and were similar in axenic and non-axenic duckweed-covered systems, suggesting that heterotrophic uptake of smaller organic compounds by duckweed was not important. Degradation of organic material was enhanced by duckweed through both additional oxygen supply and additional surface for bacterial growth. The structure of attached bacterial

communities and the manner in which oxygen was introduced appeared important, because the influence of the living duckweed community could not be simulated satisfactorily by artificial surfaces for bacterial growth, by oxygen pumps or by a combination of both.

Al-Nozaily et al. (2000) investigated the effect of depth, mixing intensity and sewage concentration on the COD removal, DO levels and the pH of duckweed sewage lagoons. Laboratory scale experiments were performed in a non-continuous batch reactor system with 0.8-41.2 l of domestic sewage exposed to constant light intensity, temperature and humidity. The treatment performance of duckweed (*Lemna gibba*)-covered sewage lagoons was studied within a COD range of 200-500 mg/l (113-294 mg filterable COD/l), in 10, 30, 70 and 95 cm deep reactors, and liquid mixing intensity (power dissipation) of 0, 0.3, 1.0, 2.3 and 34.1 W/m^3. The duration of each experiment was 20 days with biomass harvesting every 5 days. Removal of COD did not differ in duckweed-covered and control reactors. The role of duckweed cover was marginal in changing the redox potential or the DO concentration. COD removal correlated strongly with initial surface load. Concentration removal was also proportional to initial COD concentration. For a given COD mass input, increasing the depth up to 1 m affected lagoon performance only by increasing surface load, and not by hampering oxygen transfer. Mixing (up to $2.3W/m^3$) raised COD removal. Therefore, at depths beyond 70 cm, moderate mixing was recommended. The first-order kinetic removal rate coefficient for COD was $0.04\text{-}0.06d^{-1}$.

El-Kheir et al. (2007) inoculated *Lemna gibba* into primary treated sewage water systems (from the collector tank) for aquatic treatment over eight days retention time period under local natural outdoor conditions. Samples were taken below duckweed cover after every two days to assess the plant's efficiency in purifying sewage water from different pollutants and to examine its effect on both phytoplankton and total and fecal coliform bacteria. Data revealed that the duckweed mat effectively reduced BOD by 90.6% (reduced from 320 mg $O_2.L^{-1}$ at zero days reaching 30 mg $O_2.L^{-1}$ after 8 days treatment) and COD by 89% (reduced from 800 mg $O_2.L^{-1}$ to 88 mg $O_2.L^{-1}$).

1.1.3.4 Integrated model for nutrient removal

Bal Krishna & Polprashert (2008) developed an integrated kinetic model for organic and nutrient removal by duckweed-based wastewater treatment (DUBWAT) system. Four pilot-scale DUBWAT units, made of concrete blocks, were operated under ambient conditions (temperatures 30-36°C) in three different phases to determine the optimum hydraulic retention time (*t*), organic loading rate (OLR) and stocking density of duckweed (SD). The maximum COD, BOD_5 (5 days), NH_3-N, TN and TSS removal efficiencies of 84, 88, 68, 58 and 87%, respectively, were found at optimum operating conditions of *t* of 10 days, OLR of 50

kgCOD/(ha-d) and SD of 0.5 kg/m^2. The nitrogen uptake rate by duckweed was found to be 0.62 g-N/(m^2-d). An integrated kinetic model consisting of *t*, OLR, SD and temperature was developed for the DUBWAT system, based on the following plug flow equation developed by Reed & Brown (1995), which took only retention time into consideration:

$$\mathrm{Ln}(C_e/C_o) = -k_T t \qquad (1.1)$$

$$k_T = k_{20}\theta^{(T-20)} \qquad (1.2)$$

Where C_e = effluent concentration (mg/L), C_o = influent concentration (mg/L), k_T= temperature dependent, first-order rate constant (d^{-1}), k_{20} = first-order rate constant at 20°C (d^{-1}), *t* =hydraulic retention time (d), T = temperature (°C). Value of θ is 1.05.

The results obtained from the three experimental phases were used to determine the kinetic constant (k_T) by using the first-order plug-flow model (Eq. 1.1). The k_T values were calculated from the COD, BOD_5, NH_3-N and TN removal efficiencies, and the k_{20} values were determined from Eq. 1.2. Since the parameters OLR and SD were found from this study to affect the DUBWAT performance, they were included in the k_{20} model as shown in the following equation:

$$k_{20} \propto f(\mathrm{OLR}, \mathrm{SD}) \qquad (1.3)$$

where k_{20} = as defined in Eq. (1.2), OLR or λ = organic loading rate (kgCOD/(ha-d)), SD or β = stocking density (kg/m^2).
A relationship among k_{20}, λ and β was proposed as shown in the following equation:

$$k_{20} = k'\lambda^x\beta^y \qquad (1.4)$$

where *k*' = specific constant for organic or nutrient removal (unit less), *x* = reaction constant for OLR (unit less), *y* = reaction constant for SD (unit less), λ and β are as defined previously.
Eq. (5) can be converted into a linear form as shown in the following equation:

$$\ln(k_{20}) = \ln(k') + x\ln(\lambda) + y\ln(\beta) \qquad (1.5)$$

The values of *k*', *x* and *y* were determined from regression analysis (SPSS 11.5 program) using the experimental data obtained from the three experimental phases.

By combining Eqs. 1.1, 1.2 and 1.4, an integrated kinetic model is given in the following equation:

$$\mathrm{Ln}(C_e/C_o) = -k'\lambda^{x}\beta^{y}\theta^{(T-20)}t \quad (1.6)$$

The experimental data shown in from the three experimental phases were used to determine the k_T values for COD, BOD_5, NH_3-N and TN removal for each of the experimental conditions employed, using Eq. 1.1, while the k_{20} values were determined from Eq. 1.2. The constant values *k*', *x* and *y* were determined from Eq. 1.5 using SPSS 11.5 regression analysis program. The k_T values and integrated kinetic models of the DUBWAT system for COD, BOD_5, NH_3-N and TN were reported as follows, with correlation coefficient values above 0.80:

For COD removal:

$$k_T = 0.084\lambda^{-0.189}\beta^{-1.023}\theta^{(T-20)} \quad (1.7)$$

$$\mathrm{Ln}(C_e/C_o) = -0.084\lambda^{-0.189}\beta^{-1.023}\theta^{(T-20)}t \quad (R^2 = 0.81) \quad (1.8)$$

For BOD_5 removal:

$$k_T = 0.116\lambda^{-0.258}\beta^{-1.095}\theta^{(T-20)} \quad (1.9)$$

$$\mathrm{Ln}(C_e/C_o) = -0.116\lambda^{-0.258}\beta^{-1.095}\theta^{(T-20)}t \quad (R^2 = 0.81) \quad (1.10)$$

For NH_3-N removal:

$$k_T = 0.120\lambda^{-0.407}\beta^{-1.886}\theta^{(T-20)} \quad (1.11)$$

$$\mathrm{Ln}(C_e/C_o) = -0.120\lambda^{-0.407}\beta^{-1.886}\theta^{(T-20)}t \quad (R^2 = 0.82) \quad (1.12)$$

For TN removal:

$$k_T = 0.173\lambda^{-0.553}\beta^{-1.644}\theta^{(T-20)} \quad (1.13)$$

$$\mathrm{Ln}(C_e/C_o) = -0.173\lambda^{-0.553}\beta^{-1.644}\theta^{(T-20)}t \quad (R^2 = 0.80) \quad (1.14)$$

The integrated kinetic models were validated with high correlation with data obtained from literature, hence their applicability in the design and operation of the DUBWAT system.

1.1.3.5 Removal of heavy metals and heavy metal toxicity

Zayed et al. (1998) found that under experimental conditions, duckweed proved to be a good accumulator of cadmium (Cd), selenium (Se), and copper (Cu), a moderate accumulator of chromium (Cr), and a poor accumulator of nickel (Ni) and lead (Pb). The toxicity effect of each trace element on plant growth was in the order: Cu > Se > Pb > Cd > Ni > Cr. The author concluded that duckweed shows promise for the removal of Cd, Se and Cu from contaminated wastewater since it accumulates high concentrations of these elements and the growth rates and harvest potential make duckweed a good species for phytoremediation.

El-Kheir et al. (2007) found that all detected heavy metals were progressively reduced after an 8d treatment period with *Lemna gibba*; there was 100% copper and lead removal after 8 days, and the system efficiently reduced the content of zinc by 93.6%, barium by 93% cadmium by 66.7%, cobalt by 15.8%, iron by 11.8%, manganese by 10.6%, molybdenum by 25% and vanadium by 16.7%.

1.1.3.6 Effect of mat density

Under suitable conditions, duckweed can continuously develop, covering large water areas, unless the available water surface is limited. Indeed, on saturated water surfaces, the aquatic equilibrium is markedly modified since no light rays can pass through the dense plant mat. The growth of any given species is known to be governed by the size of its population (Monette et al., 2006).

Numerous mathematical models have been developed to describe plant growth, most of them being based on Michaelis-Menten kinetics. In these models, growth is regarded as being a first-order function of plant mat density. The specific growth rate is correlated to temperature, light intensity, some inhibiting compounds, biomass age, concentrations of nutrients N and P, as well as to the chemical oxygen demand (COD) (Aseada et al., 2000; Boniardi et al., 1994). In these studies plant mat density is either considered constant or devoid of any effect on plant growth. Duckweed mat density plays a crucial role since a high density seems to hinder duckweed growth (Körner & Vermaat, 1998). Monette et al. (2006) developed a more comprehensive mathematical model taking into consideration the effect of plant mat density on the growth of *Lemna minor* under controlled operating conditions; 12.5h a day light exposure and 342 $mol.m^{-2}.s^{-1}$ light intensity at 20°C. The plant growth was carried out in Hoagland medium for 7 days without harvesting. The results revealed a maximal biomass growth rate of 88 $g\text{-}dry.m^{-2}$ (1470 $g\text{-}wet.m^{-2}$) at an optimal initial mat density of 45 $g\text{-}dry.m^{-2}$ (750 $g\text{-}wet.m^{-2}$), with removal rates for nitrogen (N) and phosphorus

(P) of 483 mg-N.m^{-2}.d^{-1} and 128 mg-P.m^{-2}.d^{-1}, respectively. The intrinsic growth rate, r_i, dependant on operating conditions such as temperature, light intensity, light exposure time, presence or absence of inhibiting agents and organic matter was experimentally determined as 0.29.d^{-1} under the conditions of this study. The results indicate that the first-order growth rate (r) was dependent on the initial duckweed mat density and decreased when the density increased. A mathematical model that takes into account the mat density was developed in order to simulate the growth of *Lemna minor* under controlled eutrophication, using a second order function. Based on experiments carried out, the model exhibits a reliability of 89%. This model remains to be validated at the full-scale level.

1.1.3.7 Sulphur volatilisation

An additional positive feature of duckweed ponds may be that the duckweed cover reduces the emission of gases, such as H_2S, as compared to regular ponds. A reduction in the emission of H_2S from ponds is important, since sulphide present in the atmosphere causes odour nuisance at very low concentrations. Reported values at which atmospheric concentration of sulphide causes odour nuisance range from 0.001 to 0.2ppm (Parsons et al., 2000). The actual atmospheric sulphide concentration is affected by a number of factors, such as:

- The atmospheric conditions (Parsons et al., 2000).
- The emission rate of sulphide from the liquid phase to the atmosphere.
- The pH of the wastewater, which determines the fraction of total sulphide present as volatile unionised sulphide (H_2S).
- The influent sulphate concentration. This determines the amount of sulphide that can be formed in an anaerobic (pond) reactor.
- Biological and chemical conversion of sulphide.

H_2S is subject to biological conversion by various groups of bacteria: (1) aerobic chemolithotrophic conversion by, e.g. *Beggiatoa* and *Thiobacillus*. (2) Anoxygenic photosynthetic processes performed by purple sulphur bacteria (e.g. *Chromatium*) and green sulphur bacteria. Chemical oxidation is, in contrast to biological oxidation, found to start with the oxygenation of HS^-. The rate depends on the sulphide concentration, the availability of oxygen and the pH. The rate of biological oxidation has been reported to be a factor 10^3-10^5 times higher than chemical oxidation under tested circumstances. Kerstens et al. (2009) studied the effects of a duckweed (*Lemna gibba*) cover on the surface of waste stabilisation ponds on sulphide emissions in a laboratory scale set-up of an anaerobic pond-reactor, followed by two algae pond reactors and two duckweed pond-reactors. The concentrations of various S-components were measured at different depth in the reactors, while sulphide emissions were measured at the surface. Presence of a duckweed cover on

the anaerobic pond-reactor resulted in a 99% reduction in sulphide emission. The duckweed cover reduced H_2S volatilization via two mechanisms; by forming a physical barrier and by providing attachment area for sulphide oxidising bacteria. Colourless sulphur bacteria (*Beggiatoa* sp.) were observed on the duckweed roots. In algae pond-reactors, sulphide emissions were negligible through chemical and biological conversion of sulphide. In the presence of a physical barrier, sulphide is converted to sulphate as a result of chemical and/or biological conversion processes. The relative importance of each mechanism depends on the DO level, pH and the characteristics of the physical barrier, that is, its physical effectiveness and its surface area for bacterial attachment. The ambient pH likely affects the relative importance of chemical and biological processes, since it governs the concentration of HS^- (subject to chemical oxidation) and H_2S (subject to biological oxidation).

1.1.3.8 Removal of faecal coliforms and pathogenic microorganisms

Faecal coliform bacteria are known as one of the most important indicators of potential public health hazard due to faecal pollution. Dewedar & Bahgat (1995) studied the comparative survival of total and faecal coliform bacteria in a stable waste-water retention reservoir. The surface of the water reservoir was covered with *Lemna gibba* L. Sets of dialysis sacs were suspended in a site exposed to the sunlight, while other sets of sacs were suspended beneath the thick green layer of *L. gibba;* where sunlight was almost absent. Faecal coliform cells in dialysis sacs exposed to sunlight showed a die-off pattern with a calculated decay rate of $0.1768h^{-1}$. Faecal coliform in sacs suspended under the L. *gibba* layer did not decline during the period of the experiment.

El-Shafai et al. (2007) evaluated the nutrient and faecal coliform removal in a pilot-scale wastewater treatment system which comprised a of 40-l UASB (up-flow anaerobic sludge blanket) reactor (6h HRT) followed by three duckweed ponds in series (total HRT 15 days). The system achieved 99.998% faecal coliform removal during the warm season with final effluent containing 4×10^3 cfu/100 ml. The system was deficient in the removal of faecal coliforms during the winter, producing effluent with 4.7×10^5 cfu/100 ml. The authors hypothesized that the duckweed controlled the count of faecal coliforms in the ponds through two main processes; firstly, the recovery of nutrients from the pond may have caused a deficiency in these nutrients, secondly, the adsorption of the faecal coliforms to the duckweed followed by harvesting might have played a role in faecal coliform removal. El-Kheir et al. (2007) found that total and faecal coliform counts decreased gradually with increasing treatment period reaching minimum values of 147×10^3 and 96×10^3 CFU.100 ml^{-1}, respectively after 8 days in a duckweed treatment system with a reduction of 99.8% for both bacterial types. Ran *et al*. (2004) also carried out a pilot study on constructed wetlands using duckweed for treatment of domestic primary effluent to be used for reuse purposes.

Their results indicated that the system efficiently reduced faecal coliform by approximately 95% under average hydraulic residence time of about 4.26 days. The results from these three studies contradict those reported by Dewedar & Bahgat (1995), supporting the theory of El-Shafai that other mechanisms for faecal coliform removal exist in duckweed ponds independent of sunlight. Duckweed lacks extensive root systems onto which significant numbers of micro-organisms could become attached, and they also decrease sunlight below the duckweed mat; therefore, the removal of micro-organisms in duckweed-covered ponds is likely the result of sedimentation (Falabi et al., 2002)

Islam et al. (1990) reported that *L. minor* might serve as an effective environmental reservoir for *Vibrio cholera*.

The influent and effluent of a pond covered with duckweed with a 6 day retention time was tested for *Giardia* cysts, *Cryptosporidium* oocysts, faecal coliforms and coliphage (Falabi et al, 2002). The average number of *Giardia* cysts 15 cysts.l^{-1} in the influent and 0.35 cysts.l^{-1} in the effluent, resulting in an average reduction of 98%. On average, *Cryptosporidium* oocysts decreased by 89%, with an average number of 1.58 oocysts.l^{-1} in the influent and 0.17 oocyst.l^{-1} in the effluent. Total coliforms were reduced by 61%, faecal coliforms by 62% and coliphages by 40%. The duckweed pond was therefore more effective in reducing the number of protozoan parasites than indicator bacteria or coliphages. There was a significant correlation between the removal of *Giardia* cysts and *Cryptospordium* oocysts by the pond ($P < 0.001$). Influent turbidity and parasite removal were also significantly correlated (*Cryptosporidium* and turbidity, $P=0.05$; *Giardia* and turbidity, $P=0.01$). There was no correlation between *Giardia*, *Cryptosporidium*, coliform bacteria and coliphage removal, and the water pH and temperature. The removal of microorganisms in the pond appeared to be related to the size of the organisms, with larger organisms most likely settling to the bottom of the pond, while removal of smaller bacteria and coliphages in the pond was not as effective. The authors concluded that additional retention time could increase the removal capability of these systems, allowing more time for the micro-organisms to settle before the water reaches the outlet of the pond.

1.2 Algal-based waste stabilisation pond systems

Wastewater treatment in stabilization ponds mainly results from settling and complex symbiosis of bacteria and algae where the oxidation of organic matter is accomplished by bacteria in the presence of dissolved oxygen supplied by algal photosynthesis and surface re-aeration (Beran & Kargi, 2005).

1.2.1 Diurnal cycles of variation

The natural processes of stabilising organic waste by bacterial oxidation and that of producing oxygen by algae through photosynthesis are fundamental in the treatment of sewage by WSPs. The respiratory oxygen required by aerobic bacteria for assimilation of organic materials is met by algae photosynthetic oxygen without the need for additional aeration. In WSPs oxygen tension is an operational parameter that shows a great deal of daily and hourly variation. The rate of oxygen production is a function of the concentration of algae and other forcing functions. The growth of algae is light- and temperature-dependent, and the rate of oxygen production (photosynthetic) follows the same pattern. Temperature is a parameter that shows a marked seasonal and daily variation in WSPs. It influences photosynthesis, growth of microorganisms and bio-decomposition of organic carbon in the system. The fluctuation of pH influences the kinetics of microbial growth, species competition and product formations in the pond. Each microbial species can grow within a specific pH range which typically extends over 3-4 pH units with optimum growth rate at near the mid-point of the range. The diurnal pH change in the ponds is usually followed by net algal uptake of CO_2 during the daylight via photosynthesis and the increase of CO_2 during the night due to total bacteria and algae respiration. An increase in the pH of WSPs of up to 11 in the late afternoon is not uncommon.

Kayombo et al. (2000) used the long term data collected from secondary facultative waste stabilisation ponds (SFWSP) to determine the manner in which, pH, temperature and light intensity influence the production and utilization of dissolved oxygen. A model was formulated, and was modified to include the influence of pH and carbon dioxide. The forcing functions to the DO model were light intensity, carbon dioxide, temperature and pH. The model revealed that all forcing functions simultaneously affect the rate of photosynthesis based on the multiplicative function. The model was calibrated and validated by using the average daily data from the SFWSPs. The model yielded a linear regression coefficient of 0.87 during calibration and 0.78 during validation. Based on the model results the rate of production of DO with relation to dry algal biomass was 1.599 mg DO:mg dry weight, which is equivalent to 35.905 mg DO:mg chlorophyll-a. Such correlation between the observed data and model prediction indicates that the assumption inherent in the mathematical model formulation of the processes is valid for the description of DO production and usage in the ponds. It also suggests that, for a balanced system, the amount of DO produced by the photosynthesis process is enough to keep the system healthy. Based on the model the leading process of oxygen utilization was due to total respiration.

In 2002, Kayombo et al. investigated diurnal fluctuations of pH, dissolved oxygen (DO), water, air temperature and sunlight intensity in the waste stabilization ponds at the University of Dar es Salaam. The variation of these parameters followed the diurnal pattern of light intensity. The rate of oxygen production based on first order linear regression analysis was between 0.02 and 0.36 $mg.l^{-1}$ per h with a high production rate being observed in the secondary facultative ponds. The rate of utilization of dissolved oxygen (total respiration) during the night by the microbial population in the pond ranged between 0.016 and 0.435 $mg.l^{-1}$ per h. The average rate of increase of pH during the day was 0.0006–0.243 units of pH per h, and the rate of decrease was 0.0003–0.101 units of pH per h. The pond which received high organic loading had relatively low diurnal variations as was observed in primary facultative pond; the ponds receiving low organic loading showed a high diurnal variation of physical-chemical parameters. The relationship between average hourly DO and pH followed a polynomial trend with the coefficient of regression (R^2) ranging from 0.76 to 0.82. It was concluded that the diurnal variation of the parameters in the WSPs was due to hourly and daily variations in the light intensity. The authors also concluded that the pH levels in the pond system may be used as a performance indicator. A pH above 8 was produced by photosynthetic rate that demanded more CO_2 than quantities replaced by respiration and decomposition, and a pH level below 8 indicated the failure of photosynthesis to completely utilize the CO_2 produced, and thus indicated the presence of a high concentration of CO_2. At a pH above 8, the ammonia concentration became high and thus affected the photosynthesis activity as it is toxic to algae. Carbon dioxide in the pond may limit algae activities when the rate of oxidation of organic matter is preceded by high uptake of carbon dioxide by algae. It appeared that this phenomenon occurred when the pH in the water was high (more than 8).

Wastewater stabilization ponds, although often only 1-2 m deep, stratify and destratify intermittently depending primarily on weather conditions. Stratification can be observed in vertical profiles of water temperature, dissolved oxygen, pH and other water quality parameters. In three stabilization ponds of a small Minnesota town, Gu & Stefan (1995) observed that stratification developed primarily by differential heating of the pond water through its surface and, in the absence of artificial aeration or mixing devices, by insufficient wind mixing. The resulting water temperature stratification affected other parameters in a variety of ways through chemical, microbial and planktonic kinetics and reduced vertical mixing. To gain a better understanding of stabilization pond water quality dynamics, the authors monitored temperature profiles at 20-min intervals in three WSP systems, and a dynamic lake water quality model was modified and applied to simulate the temperature stratification. A 12-h time step option was incorporated into the program in order to capture the diurnal variation in stratification. The level of agreement between field measurements

and numerical simulations demonstrated that water temperatures and stratification dynamics in a shallow and small pond could be simulated on a diurnal timescale with a standard error from 1.0 to 1.5°C between simulation and measurements. The temperature stratification dynamics in these ponds were impressive in terms of the occasional strength of the stratification stability as well as its rapid variability in time. Vertical temperature differentials of up to 8°C over the first meter of depth were observed and simulated. Variations in surface temperatures (0.04 m depth) from 6:00 a.m. to 6:00 p.m. also reached about 8°C. The model included wastewater inflow in the form of a vertical jet and water transfer between ponds in the form of non-surface inflow and outflow.

1.2.2 COD removal

Anaerobic waste stabilization ponds (AWSPs) play a major role in the treatment of wastewaters containing high suspended solids and organic concentrations. The underlying treatment mechanism of the AWSPs is anaerobic digestion, a process which has been subjected to intensive investigation to reveal the complex reactions and to understand the limitations involved. Digestion of complex organic material in an anaerobic environment can be briefly described in three sequential steps. In the first step, hydrolytic fermentative bacteria hydrolyse the complex polymeric substrates such as carbohydrates, proteins, and fats to simpler organic end products including aldehydes and alcohols but principally volatile organic acids. In the second step, hydrogen-producing and acetogenic bacteria convert the fermentation products of the first decomposition-step into hydrogen, acetate and CO_2. In the third step, methanogens convert acetate, hydrogen and CO_2 into CH_4. The gaseous end products released in the final stage of organic digestion may escape from the liquid in the form of H_2S, CO_2 and CH_4, or remain in the liquid to serve as a buffering system like NH_3 and CO_2 (Toprak, 1995 (2)).

Toprak (1995 (1)), operated two laboratory-scale AWS columns (59l each) using domestic wastewater in a repeated batch model. Data from one of the reactors was used to develop a dynamic model to describe the removal of soluble chemical oxygen demand (SCOD). This model was applied to the other reactor data to test its reliability, and was found to be appropriate. The rate constant for SCOD removal and temperature correction coefficient were 0.325 g $SCODg^{-1}$ MLVSS (mixed liquor volatile suspended solids) day^{-1} (for 20°C) and 1.017, respectively. The author concluded that the minimum hydraulic retention time in an AWSP should be more than 4 days, and for the land-scale application of an AWSP, the SCOD loading rate must be lower than 0.15 kg $SCODm^{-3}.day^{-1}$. The methane production rate was found to be 0.29 m^3 CH_4 kg^{-1} $COD_{removal}$.

In a full-scale study, Toprak (1995 (2)) measured CH_4 and CO_2 emission rates in an AWSP in Portugal and correlated these with the removed organic loading rate, and ambient air temperature. Strong correlation was found between biogas composition and removal efficiency. Correlation coefficients for CH_4 and CO_2 were 92.6% and 76.9%, respectively. The lowest liquid temperature at which the AWSP served as a primary sedimentation basin without any biological degradation, was found to be 11.7°C, with a total COD removal of 30%. The first-order COD removal rate constant and temperature correction factor were 0.221 day^{-1} and 1.117, respectively. Biogas production rates varied between 28.68 and 83.171 $m^2.day^{-1}$. Average volumetric biogas production rate was 19.57 $l.m^{-3}.day^{-1}$. Biogas volume changed during the day and 24h-averaged value was 2.02 $lm^{-2}.h^{-1}$. Biogas conversion ratio was between 0.160 and 0.702 m^3 k $COD^{-1}_{removed}$. Percentages of CH_4 and CO_2 found in the biogas were between 52-80%, and 7-28%, respectively. CH_4 production rates varied between 18.18 and 48.74 $l.m^{-2}.day^{-1}$ depending on influent COD loading and liquid temperature. CO_2 formation rates were between 3.08 and 9.79 $l.m^{-2}day^{-1}$. Mean CH_4 and CO_2 production were determined to be 82.34 and 14.40 m^3day^{-1}, respectively. Volumetric production rates for CH_4 and CO_2 were obtained to be 13.54 and 2.37 $l.m^3day^{-1}$, respectively.

1.2.3 Removal of pathogenic microorganisms

1.2.3.1 Protozoan cysts and helminth eggs

One of the main advantages of waste stabilization ponds is their capacity to remove pathogenic organisms. Protozoan cysts and helminth eggs are removed mainly by sedimentation, and ponds are generally able to produce effluents with concentrations close to or equal to zero (Von Sperling et al., 2005). Over a 24-month period, Amahmid et al. (2002) analysed grab samples from a pilot stabilization pond system in Marrakech for the presence of *Giardia* cysts and *Ascaris* eggs. *Giardia* cysts were detected in 50% of raw wastewater samples with an average concentration of 2.8 x 10^3 cysts/l, while *Ascaris* eggs were isolated in 39.3% of samples with a mean number of 1.7 eggs/l. The concentration of cysts and eggs varied according to seasons, with highly significant concentrations during the hot, dry periods (Spring and Summer). Enumeration of *Giardia* cysts and *Ascaris* eggs in the sediment at the entrance of the system resulted in average numbers of 1.3 x 10^3 cysts/g and 29.6 eggs/g dry weight of sediment. These concentrations decreased towards the outlet of the ponds, where the sediment was free of *Giardia* cysts and *Ascaris* eggs, suggesting an association between eggs and cysts settlement and settleable solids. At the outlet of the system, neither *Giardia* cysts nor *Ascaris* eggs were found in treated wastewater. In general, it would be considered preferable for potentially pathogenic organisms to partition into the sludge, which can be further treated before reuse or disposal, than in the effluent which may

be discharged into a watercourse which may then be subsequently used for domestic, agricultural or recreational purposes or abstracted for potable supply.

Oraki et al. (2001) determined the reduction in the infectivity of *Cryptosporidium parvum* oocysts after exposure to the physicochemical conditions of high-rate algal ponds in semi-permiable bags, using a neonatal mouse infectivity model. They found a 97% reduction in cyst infectivity after a three day retention time in the ponds. The semi-permeable bags used in the experiments excluded predation, bacterial or fungal infection, and the effect of large molecules as potential factors for oocyst inactivation. The lower conductivity values for the bags suggest that only small molecules were able to diffuse through the membrane, leaving temperature, pH, small ions (ammonia, phosphates, etc.), and light as the main factors potentially responsible for the significant reduction in oocyst infectivity. The authors concluded that the conditions of pH, ammonia, and/or light seemed to be the major factors for the inactivation of the oocysts in wastewater. Algal-based water treatment systems therefore allow not only for cyst removal, but also their inactivation as a result of their associated physicochemical conditions; high pH, high dissolved ammonia concentration as a result of the high pH, and exposure to sunlight.

1.2.3.2 Pathogenic bacteria and viruses

Pathogenic bacteria and viruses are removed by a combination of various factors that lead to an unsuitable environment for them, including high pH, high DO, ultraviolet radiation, predation, and starvation. Sunlight exposure is considered to be the most important cause of ``natural'' disinfection in WSPs.

Photosynthetically active radiation, or PAR, falls between 400 and 700nm. However, bactericidal sunlight can have a wavelength of anything from 290 to over 700nm. Ideally therefore, knowledge of the aquatic optics of bactericidal light should encompass the penetration of a range of wavelengths between 290 and 700nm. The light absorption properties of natural waters are attributable to four components: the water, gilvin (dissolved yellow matter), algae and tripton (inanimate particulate matter). The total absorption at a given wavelength can be obtained by adding together the absorption of these four separate components. Although apparently colourless, water absorbs light moderately well at wavelengths <550nm. Gilvin absorbs strongly in the UV spectrum and is known to be present in sewage and WSP. Algae, being photosynthetic, have large quantities of pigments, which also impede light penetration. In productive waters, such as WSP, light absorption by algae may be very important, often limiting the growth of the algae themselves. The absorption spectra of algal cells largely reflect the absorption of the photosynthetic pigments, with peaks at around 440 and 680nm. Changes in the attenuation coefficient at a given

wavelength associated with a change in algal biomass will reflect the absorbance of the algae at that wavelength. The relationship between the two is approximately linear, with the absorbance due to algae being related to the amount of chlorophyll a (an indicator of algal biomass) by a constant Kc. Scattering is caused both by the water itself and particles, such as bacteria, within the water. The latter predominates in all natural waters. Scattering increases the attenuation coefficient of a body of water by increasing the path length of the photons, and light is not "used up" in any way.

Curtis et al. (1994) studied the penetration of light into waste stabilization ponds because of its importance in pathogen removal and algal productivity. The objective of the study was to characterize the fundamental aspects of WSP optics and to discover the nature and cause of spectral and inter-pond variations in light penetration. The authors found that the attenuation of light in ponds was dominated by light absorption by gilvin and algae, with light scattering processes (turbidity) being of no importance. Gilvin exerted a strong influence over the spectral variation, and longer wavelengths penetrated much better than short wavelengths. Estimates of attenuation coefficients in the UV spectrum suggested that these wavelengths are less penetrating than previously reported. Differences in algal concentrations caused the differences in light attenuation seen between ponds, and it was noted that short wavelengths were more affected by changes in algal biomass than long ones. In the absence of algae there appeared to be a lower limit to the clarity of ponds dictated by gilvin and other substances. Secchi disks were found to be reliable instruments for measuring light penetration.

Davies-Colley et al. (1999) examined the influence of dissolved oxygen (DO), pH, and particulate and dissolved constituents in WSP effluent, on sunlight inactivation of faecal micro-organisms, using small reactors operated under controlled physico-chemical conditions. Inactivation of both enterococci and F-RNA phages increased strongly as DO was increased, and also depended on light-absorbing pond water constituents, but pH was not influential over the range investigated (7.5 to 10). Inactivation of *E. coli* increased strongly when pH increased above 8.5, as well as being strongly dependent on DO. Inactivation of F-DNA phage was independent of the factors investigated. These results are consistent with the F-DNA phages being inactivated as a result of direct DNA damage by UVB in sunlight, whereas the other three microbiological indicators are inactivated as a result of photo-oxidative damage, although the target of damage is apparently different. Our findings of diverse influences of physico-chemical conditions suggest difficulties in interpreting data for a single micro-organism to indicate WSP effluent quality. However, sunlight remains the factor of over-riding importance, and disinfection in WSPs may be enhanced by increasing sunlight exposure

The decay of coliforms (thermotolerant coliforms, or more specifically *Escherichia coli*) in ponds is, from a practical point of view, accepted as being able to represent satisfactorily well the removal of pathogenic bacteria and, under many circumstances, viruses (Von Sperling et al., 2003). Modelling of the decay of coliforms in ponds is therefore important as a means of predicting the suitability of the effluent for reuse (agriculture or aquaculture) or discharge into water courses. Generally speaking, ponds designed for the removal of coliforms to such low levels as to comply with WHO guidelines (WHO, 1989) for unrestricted irrigation (≤1000 MPN/100 mL) require long retention times that make them likely to achieve, under normal conditions, helminth eggs counts that also comply with these guidelines (≤1 egg/L). Thus, the removal of coliforms is usually the controlling factor in the assessment of the quality of the pond effluent and its potential for further use or discharge.

From an operational perspective, after commissioned, ponds have very few or no way of controlling the quality of the effluent. Consequently, the design stage is of foremost importance in defining the likely quality of the final effluent. The most important application of mathematical models for ponds is therefore in assisting the development of the most suitable design criteria for the conditions under analysis. For a model to be used for design purposes, it needs to rely on input variables that are obtainable, measurable or verifiable (von Sperling, 2005).

Coliform die-off in ponds is usually modelled assuming first-order kinetics (die-off rate directly proportional to the concentration). There are basically three models to represent the reactor hydraulics: plug flow, complete mix (also CSTR-completely stirred tank reactor) and dispersed flow. The dispersed flow model is more flexible since it may be set to adjust to different pond geometries. Plug-flow models are indicated for more elongated ponds, while the complete-mixed model is more suited to square or mildly rectangular ponds. Von Sperling (2002) stressed the adequacy of the dispersed flow model, but presented a methodology and equations for converting coefficients derived for this model into coefficients for the complete-mix and plug-flow models. For the same removal efficiency, it was shown that the K_b value (coliform die-off coefficient) for complete mix will always be higher and the K_b value for plug flow will always be lower than the K_b for dispersed flow Depending on the hydraulic regime assumed for the pond, different formulae are available for the estimation of the effluent coliform concentration of a facultative or maturation pond.

Von Sperling et al. (1999) investigated the coliform removal in 33 facultative and maturation ponds in Brazil. The ponds were located in different parts of the country, with climates ranging from tropical to subtropical and latitude from 7 to 24°S. The ponds had different physical configurations, temperature and retention times. Two flow regimes were

investigated, namely completely stirred tank reactors (CSTR) and dispersed flow. In the dispersed flow model, the pond depth (*H*) and the hydraulic retention time (*t*) were found to have a major influence on the value of the coefficient K_b. an equation based on regression analysis was derived for estimating the die-off coefficient for the dispersed-flow model, where K_b is the coliform die-off coefficient at the standard temperature of 20°C (d^{-1}); *H* the pond depth (m); *t* the theoretical hydraulic detention time (=volume/flow) (d):

$$K_b = 0.917H^{0.877}t^{0.329} \quad (1.15)$$

The utilisation of the dispersed flow K_b model for the estimation of the 66 values of the log effluent concentration of faecal coliforms in the 33 ponds gave very good prediction capability (R_2=0.959). A simple equation for the estimation of the dispersion number d, based on a rearrangement of was also derived leading to:

$$d= (L/B)^{-1} \quad (1.16)$$

where L is the pond length (m); B the pond breadth (m).

Based on the proposed model for dispersed flow, the required pond volumes and surface areas for different depths and *L*/*B* ratios were calculated. The results show that a shallow pond, due to its greater K_b coefficient, requires less surface area, for a given efficiency of coliform removal, compared to a deep pond, even though the latter has a higher retention time. Even though it was recognised that simple relationships as those from Eqs. 1.15 and 1.16 could be too crude for representing the multitude of factors that contribute to the decay of coliforms (K_b) and the pond hydrodynamics (d), the equations have the advantage of depending only on variables that are known at the design stage (*H*, *t* and *L*/*B*).

The relative sensitivity of the model given by Equations (15) and (16) to the input variables, especially the dispersion number d, was discussed by Von Sperling (2003). A set of simulations (1000 runs) was undertaken, allowing a sensitivity analysis of d, in conjunction with other coefficients and input data used in the design of facultative and maturation ponds (e.g. population, wastewater flow, coliform die-off coefficient and others).The results of the simulations suggested that, when considering the high level of uncertainty in all input variables used in the design of ponds for coliform removal, the dispersion number d does not present a greater influence on the model prediction, compared with the other input variables. Based on these considerations, it is likely that, for design purposes, simple models for the prediction of d can be used, without significantly affecting the estimation of the

effluent coliform concentration, considering the existing uncertainty in all other input variables.

This theory was tested further by von Sperling in 2005, who extensively evaluated the coliform decay in facultative and maturation ponds, based on data from 186 different ponds in the world. The ponds encompassed a very wide diversity in terms of physical and operating conditions, covering most situations encountered in practice. The median values for the coliform removal efficiencies were 1.8 log units (98% removal) for primary facultative ponds, 1.0 log units for secondary facultative ponds (90% removal) and 1.2 log units (94% removal) for each maturation pond in the series. The model presented previously by the author (Von Sperling, 1999) for estimating the coliform die-off coefficient according to the dispersed-flow regime, at the standard temperature of 20°C ($K_b = 0.917 H^{-0.877} t^{-0.329}$) and the dispersion number ($d=(L/B)^{-1}$) was validated with a subset of new independent data (153 ponds). The fitting was considered good ($R^2 = 0.909$ for the logarithm of the effluent coliform concentration). Two equations to be used for design purposes were derived for estimating the die-off coefficient K_b (dispersed flow, 20°C) in facultative and maturation ponds. The first equation led to a slightly better fitting with the observed logarithm of the effluent coliform concentrations ($R^2=0.874$), and related K_b with the pond detention time t and depth H ($K_b=0.682 H^{-1.286}\ t^{-0.103}$). The other equation also led to a satisfactory fitting ($R^2 = 0.845$), but was slightly simpler, depending only on the pond depth ($K_b = 0.549 H^{-1.456}$).

Oragui et al. (1993) monitored a series of ten waste stabilization ponds (a 1d anaerobic pond followed by nine 2d ponds) for the removal of *Vibrio cholerae* O1. The anaerobic pond reduced the mean number of V. *cholerae* from 485 per litre of raw wastewater to 28, and there was then a very gradual removal in the next five ponds, after a cumulative retention time of 11d, to zero.

1.2.3.3 Predictive model to determine facultative WSP effluent quality

Beran & Kargi (2005) developed a dynamic mathematical model to predict the effluent quality of facultative wastewater stabilization ponds. For a sound representation of sediment-water column, water column-atmosphere interactions and stratification due to variations in dissolved oxygen concentrations, a two-dimensional hydraulic model was employed considering dispersed flow and diffusion in horizontal and vertical directions, respectively. The resulting partial differential equation system was solved using finite difference methods and matrix manipulation techniques. The model was calibrated and evaluated on the basis of collected data from a full-scale facultative stabilization pond in Selşuk, Izmir in Turkey. Variations of COD, NH_4-N, PO_4-P, dissolved oxygen, bacteria and algae concentrations with time and the dimensions of the pond were estimated by using the

dynamic model. COD concentration increased in the middle of the pond because of high mid-day COD load which gradually decreased as a result of biodegradation and dispersion towards the end of the pond. COD concentration increased with increasing depth because of low dissolved oxygen levels at lower layers. Effluent COD levels varied between 100 and 300 $mg.l^{-1}$ ($g.m^{-3}$) depending on the variations in the COD loading rate. Bacteria concentration increased slightly with the length of the pond as a result of low microbial growth with a low retention time and plug-flow behavior. Bacteria concentration decreased with the depth of the pond because of dissolved oxygen limitations beyond the depth of 0.2 m. Effluent bacteria concentration varied between 20 and 70 $mg.l^{-1}$ ($g.m^{-3}$) and increased with the time of operation because of increasing water temperature. *Chlamydomonas* sp. and *Euglena* sp. were the most abundant algae in the modelled ponds. A small number of the diatom *Navicula* sp. was also observed. Algae were present only at the surface layer of the pond and were highly motile except *Navicula* sp. As a result of low levels of light penetration because of water turbidity, algae concentration dropped sharply for the pond depths above 0.2 m. Influent algae concentrations and growth rate increased in spring because of increasing temperature and improved light availability. Dissolved oxygen concentration (DO) was considered as one of the most important parameters determining COD and nutrient (N, P) removals. Effluent dissolved oxygen concentrations varied between 4 and 0.5 $mg.l^{-1}$ ($g.m^{-3}$) depending on the influent COD concentrations. The DO concentration decreased with time because of increasing bacteria concentrations towards the end of sampling period. DO decreased with the depth of the pond, because there was no algal oxygen production or surface aeration at the depths above 0.2 m and DO diffusion through water column could not compensate for bacterial respiration. DO also decreased with the length of the pond are due to increasing bacteria concentrations. Similar to COD variations, nitrate-N increased in the middle of the pond length and then decreased towards the end of the pond as a result of varying influent concentrations. NO_3 concentrations tend to decrease when and where COD and bacteria concentrations are high, but dissolved oxygen is low as a result of denitrification. Effluent nitrate-N varied between 1 and 3 mg l^{-1} (gm^{-3}) throughout the sampling period. Ammonium-N concentrations varied between 20 and 40 $mg.l^{-1}$ ($g.m^{-3}$) and phosphate-P levels were between 2 and 10 $mg.l^{-1}$. The authors concluded that the model can be used for design of new stabilization ponds and also, for improving the effluent quality of existing ponds.

1.2.3.4 Pathogen inactivation in WSP sludge

To support the development of safe and feasible sludge management strategies, Nelson et al. (2004) studied the accumulation rates of sludge and its characteristics in four primary wastewater stabilization ponds in central Mexico (three facultative and one anaerobic). The accumulation rates and distribution of sludge were determined by measuring the thickness

of the sludge layer at 8-40 locations throughout each pond. The average, per capita sludge accumulation rates ranged from 0.021 to 0.036 m^3/person/yr. In the anaerobic pond the sludge distribution was uniform throughout the pond, whereas in the three facultative ponds most of the sludge accumulated directly in front of the inlet. In this research, from 8% to 25% of the ponds' volumes were occupied by solids, resulting in proportional decreases in the design hydraulic retention time (HRT). It is likely that the effective HRTs in the facultative ponds were even further reduced by the formation of preferential flow paths and dead zones. The results from this research contributes to a growing body of evidence demonstrating that in facultative ponds with single inlets, the majority of sludge accumulates directly in front of the inlet. More information is needed on alternative inlet configurations that would distribute the sludge over a larger area, such as installing additional inlet pipes or increasing the inlet velocity or direction. To measure the horizontal and vertical variation in the sludge characteristics, sludge cores were collected from 3 to 7 locations in three of the ponds. Each core was divided into 4 sub-samples in which various physical, chemical, and microbiological parameters were measured. In addition, the inactivation of several pathogen indicator organisms was studied in a batch of sludge for 7 months. The inactivation rates of indicator organisms were estimated and the results provide strong evidence that most bacterial pathogens are inactivated within several months in the sludge layer, whereas the inactivation of viral pathogens may take several years, depending on the initial concentrations; the inactivation of *Ascaris* eggs was even slower. Reasonable estimates of the inactivation of faecal coliform bacteria, faecal enterococci, F+ coliphage, somatic coliphage, and *Ascaris* eggs in WSP sludge in central Mexico could be made using first-order rate constants of 0.1, 0.1, 0.01, 0.001, and 0.001 d^{-1}, respectively. From the observed changes in the concentrations of total solids and the volatile to fixed solids ratio, empirical equations were developed to describe anaerobic degradation and compression which are the two most important processes affecting the volume of sludge after its deposition. These regression equations can be used to evaluate different processes for sludge removal. The rate of anaerobic degradation decreased significantly after the first year, after which the long-term, first-order inactivation rate constant ranged from 0.042 to 0.122 yr^{-1} in the different ponds.

1.2.3.5 Pond geometry and design

Natural wastewater treatment systems such as WSPs are particularly subjected to varying environmental factors of different kinds, for example, temperature, rainfall and evaporation regimes, wind speed and direction and solar energy intensity. WSP designers have some control only upon one single process variable-hydraulic retention time (HRT). However, the HRT distribution of influent wastewater volumes will be affected by some of the other factors. Pond design involves several physical, hydrological, geometrical and dynamic

variables to provide high hydrodynamic efficiency and maximum substrate utilization rates. Computational fluid dynamic modeling (CFD) allows the combination of these factors to predict the behavior of ponds by using different configurations. Abbas et al. (2006) applied two-dimensional CFD modeling on WSPs treating wastewater with various rectangular shape configurations. The two-dimensional depth-integrated model SMS was used in this study to simulate hydrodynamics and water quality. A set of 12 configurations including baffling and pond geometry was modeled. The model was run at steady state with raw wastewater to study the effect of the assumed rectangular shapes and dimensions with constant area, for various values of water depth, flow rate and hydraulic retention time (HRT) of raw wastewater. The model was also run for different rectangular shapes with baffles. The area was manipulated by increasing the ratio between rectangular width and length as one, two, three and four times, respectively. Biochemical oxygen demand (BOD), dissolved oxygen (DO) concentrations and velocities distribution were recorded for different rectangular shapes with different numbers of baffles. The results showed that the increase in pond length and width ratio caused a bulk increase in the removal efficiency of BOD, slight increase in the DO concentration and slight increase in the flow velocity. Results showed that the rectangular shape ratio ($L_1/L_2 = 4$) with the provision of two and four cross baffles at 1/3L (two baffles) and 1/5L (four baffles), respectively, most efficient to improve overall water quality.

These results agree with those of Kilani & Ogunrombi (1984), who compared the performance of three baffled laboratory-scale facultative stabilization ponds with that of an unbaffled control pond. The hydraulic characteristics of the ponds were estimated from the results of tracer tests. The removal of BOD, COD and TS for the different ponds showed that the longer retention period obtained as a result of using baffles corresponded with an improvement in the efficiency of removing organic and solid matter. The results of the tracer tests also showed that the greater the number of baffles, the closer the system was to the ideal plug flow pattern giving the best BOD removal efficiency. The biochemical oxygen demand (BOD_5) removals achieved with the control pond and with the ponds having 3, 6 and 9 baffles were 79, 81, 86 and 89% respectively and the chemical oxygen demand (COD) removals were 81, 84, 84.2 and 84.2%. The reductions in total solids (TS) were respectively 43, 46, 51 and 64%.

In 1995, Ellis & Rodrigues developed a series of multiple regression design equations which, for both facultative and maturation ponds, relate either BOD removal or the removal of faecal coliform organisms to a number of environmental and other parameters, employing the results gathered over a 22 month period for the operation of facultative and maturation ponds in the Cayman Isles. For the facultative ponds, BOD removal was principally influenced

by not only loading and retention time, but also by solar radiation and hours of sunshine, rainfall and pond depth. On the other hand, loading, retention time, pond depth and the wastewater electrical conductivity were the principal factors influencing faecal coliform removal. In the maturation ponds faecal coliform removal was influenced by the same parameters as for the facultative ponds but with BOD removal neither pond depth nor rainfall appeared to be of any importance.

1.2.3.6 Polishing of final effluent

Excessive loss of algae from waste stabilisation ponds results in a deterioration in the effluent quality. When proper hydraulic residence time is not provided for the WSPs, the content of organic matter in the effluent can be higher than that of the influent. This has been recognized as one of the most troublesome operational problems. Thus, if the system is not designed to allow sufficient hydraulic retention time, separation of the algae is essential to produce lower concentrations of BOD, suspended solids, and nutrients.

1.2.3.6.1 Water hyacinth

Kim & Kim (2000) operated pilot-scale integrated processes where water hyacinth (*Eichhornia crassipes*) ponds (WHPs) were coupled with WSPs to determine the effects of the plant and root mats on reduction of algal concentrations on a quantitative basis. They found that the WHPs reduced the amount of algal cells in the effluent significantly. The stems and leaves of the hyacinth provided shading of the pond, thus limiting the light available to the algae and resulting in their death and decay. Algal particles also attached to the surface of the plant roots. It was observed that the use of the water hyacinth for separating algal particles reduced the dissolved oxygen levels of the treated water, from between 10 and 16 mg/l in the WSP effluent to less than 3 mg/l in the final effluent of WHPs. However, the high pH (9-10) of the WSPs effluent was adjusted to 6-7 as it passed through the WHPs because of the changes in the carbon-equilibrium.

Yi et al. (2009 (1)) investigated the coupling of WSPs with WHPs as means to upgrade secondary effluent from a waste water treatment plant. Naturally-occurring nitrification and denitrification phenomena were monitored and evaluated on a quantitative basis. In nitrification and denitrification, a reduction of nitrogen is accomplished by two conversion steps. In the first step, ammonia is nitrified to nitrate. In the second step, nitrate is reduced to nitrogen gas. For nitrification to occur, each gram of ammonia nitrogen theoretically requires 4.57 g of oxygen. Denitrification requires an anoxic condition because denitrifying bacteria obtain energy for growth from the conversion of nitrate to nitrogen gas, but require a carbon source for cell synthesis. Thus, to convert each gram of nitrate to nitrogen gas,

5-9 g of carbon must be supplied. The approach for achieving nitrification and denitrification includes the creation of a series of alternating aerobic and anoxic stages which are usually established by external oxygen and carbon supplies. The WSP supplied oxygen to the post process WHP, while the inside of the WHP provided a unique denitrification environment caused by respiration of nitrifying bacteria on the surface of the hyacinth roots, and biodegradation of the algae separated by hyacinth plant roots. The nitrification and denitrification rates were 0.04 and 0.02 g/kg.day at 20°C (wet weight basis), respectively, and were strongly affected by seasonal change. Nitrification and denitrification were expected to occur as the water temperature was maintained between 20°C and 30°C. As plant density increased, their nitrification and denitrification rates also increased. The alkalinity balance corresponded fairly well with nitrogen behaviour during most of the operational period. Oxygen balance test results validated that the water hyacinth was crucial not only for separating algal particles from the WSP, but also for biological nitrogen reduction.

In 2009 (2), Yi et al. developed a dynamic model to predict nitrogen removal in WHPs receiving effluent from WSPs. The model was based on the biofilm reaction on the root surface of plant and pond walls. The model consisted of mass balances of six main substrates including: particulate organic nitrogen (PON), dissolved organic nitrogen (DON), ammonium ($NH4^{+}$), nitrite and nitrate (NO_x), soluble chemical oxygen demand (SCOD), and particulate chemical oxygen demand (PCOD). The model, incorporating major nitrogen transformation mechanisms such as hydrolysis, mineralization, and nitrification-denitrification, also accounted for carbon consumption and plant uptake. The model's application to a pilot plant showed good agreement between measured and predicted values. According to the modeling results, in the WHPs, nitrification and denitrification were the predominant nitrogen removal processes occurring simultaneously. Temperature and hydraulic retention time had a profound effect on the performance of nitrogen removal while an algae biomass (PCOD) accumulated in the WHPs, was a useful carbon source for denitrification.

1.2.3.6.2 Constructed wetlands

Senzia et al. (2003) conducted field investigations on pilot scale horizontal subsurface flow constructed wetlands (CW) units located downstream of waste stabilisation ponds (WSP). Six units were filled with gravel 6-25 mm in diameter in equal proportion, which gave an initial hydraulic conductivity of 86 $m.d^{-1}$. Four units covering surface area of 40.7 m^2 each, were located downstream of primary facultative pond, and the other two units with surface area 15.9 m^2 each were located downstream of the final maturation pond. Based on a total nitrogen inflow of 1.457 g $N/m^2.d$, *Phragmites* showed 54% removal and *Typha* 44.2%. While the system downstream of the primary facultative pond had accretion as a major pathway,

accounting for 19.1% of inflow nitrogen, denitrification was the major removal mechanism in the system downstream of the maturation pond, accounting for 20.5%. Based on the findings presented, the system located downstream of the primary facultative pond reduced BOD_5, TN, NH_3-N, NO_3-N + NO_2-N, TSS and Org-N load at rates of 4.039 g/m^2.d (82.2%), 0.823 g/m^2.d (56.2%), 0.345 g/m^2.d (38.2%), 0.034 g/m^2.d (51.5%), 8.897 g/m^2.d (91.5%) and 0.444 g/m^2.d (89.9%), respectively. The NH_3-N increased by 25.1% in the system located downstream of the maturation pond due to anaerobic mineralisation of accumulated organic nitrogen, specifically algae. However, the system reduced BOD_5, TN, NH_3-N, NO_3-N + NO_2-N, TSS and Org-N at the rate of 1.917 g/m^2.d (71.6%), 0.666 g/m^2.d (48.1%), 0.167 g/m^2.d (56.4%), 8.615 g/m^2.d (89.3%) and 0.586 g/m^2.d (70.3%), respectively.

1.2.3.6.3 Rock filters

Rock filters have been used for to remove algal solids and associated biochemical oxygen demand (BOD) in effluents mainly from primary maturation ponds (the first maturation pond following the facultative pond). Although unaerated filters were able to remove BOD and SS, they were unable to remove ammonia. In 2005, Johnson & Mara investigated rock filter aeration to determine if ammonium-N could be effectively removed in rock filters by nitrification. This would be beneficial, not only for rock filters treating pond effluents, but also for any small wastewater treatment works where upgrading is required to reduce the concentration of ammonia discharged. A pilot-scale aerated rock filter was investigated, in parallel with an unaerated control to determine whether aeration provided conditions within the rock filter for nitrification to occur. Facultative pond effluent was applied to the filters at a hydraulic loading rate of 0.15 m^3/m^3.day during the first 8 months and at 0.3 m^3/m^3.day thereafter giving a retention time of just over 1.5 days. The facultative pond effluent exceeded the required ≤60:40 mg/l 95-percentile concentrations for SS and BOD. The influent SS and BOD trends suggested that the concentrations of these parameters were largely due to the algal cells in the facultative pond effluent. The aerated filter removed >90% of both SS and BOD; its effluent BOD was consistently <5 mg/l. SS and BOD removals in the control filter were much more variable (70-90% and 45-90%, respectively); nevertheless the control filter achieved the EA requirement for both these parameters. Effluent TKN from the aerated filter was consistently <5 mg/l, whereas the control filter frequently failed to reduce TKN. A similar pattern established for ammonium removal. The NH_4^+-N concentration in the influent to both filters was reasonably similar and varied over the eight month period from 2 to 7 mg/l. The aerated filter effluent consistently removed NH_4^+-N to <2 mg/l, but the control filter did not remove any NH_4^+-N, in fact, its concentration generally increased. Nitrate was produced in the aerated filter, but not in the control. Nitrite was below detection levels in both the influent and effluent for both filters. Typical faecal coliform numbers in the facultative pond effluent were 10^5 per 100 ml in winter and 10^3 per 100 ml in

summer. Numbers were always >1000 per 100 ml in the control filter effluent, but in the effluent from the aerated filter they were consistently reduced to <1000 per 100 ml and often to <100 per 100 ml. The authors concluded that rock filters can advantageously replace maturation ponds and/or constructed wetlands for the tertiary treatment of facultative pond effluents, with a consequent reduction in land area requirements, and that the filters should be aerated if low levels of ammonia and/or faecal coliforms are required.

In 2007, Johnson et al. detailed typical UK land costs, climate and winter performance data for a pilot-scale waste stabilization pond with various upgrading technologies in order to identify the relative advantages and disadvantages of both maturation ponds and rock filters for facultative pond effluent polishing. Winter was selected as the test period as BOD and SS were high, an increase in flow was observed due to rainfall, temperatures were low, and ammonia concentrations were high. System A consisted of two tertiary maturation ponds in series; System B, two tertiary maturation ponds in series followed by a reed bed channel; System C, a control rock filter; System D, an aerated rock filter; and System E, a constructed wetland. System D, the aerated rock filter, was found to perform best, closely followed by System B, which had two tertiary maturation ponds in series. Each of systems B and D had its advantages and disadvantages. Neither required regular sludge disposal (desludging estimates for both systems are approximately every 10 years) and they both had low operation and maintenance costs. However, while aerated rock filters require mechanical aeration (initial costs plus on-going aeration costs), maturation ponds and reedbeds have higher capital costs due to the larger land area required (14 m^2/p.e. for a primary facultative pond, two maturation ponds and a reedbed channel, compared with 7.35 m^2/p.e. for a primary facultative pond and an aerated rock filter (Mara, 2006)) and the cost of additional excavation. Aerated rock filters could therefore be advantageous for small communities, not only for upgrading facultative pond effluents but for upgrading any secondary treated wastewater. They enable nitrification in winter, improved BOD and SS removal, minimal sludge production and the authors reported compliance with a 20/30/10 BOD/SS/NH_4^+-N discharge consent, even at low UK winter temperatures.

In 2007, Mara & Johnson analysed the results from their earlier studies, as well as studies undertaken by others (Neder et al. (2002); Saidam et al., (1995)) in order to determine the suitability of the effluent produced by an aerated rock filter for discharge to surface waters and for irrigation, in both temperate and tropical climates. In temperate climates, as was shown in their previous papers for studies in the UK, a hydraulic loading rate (HLR) of 0.3 m^3 of facultative pond effluent per m^3 of gross rock filter volume day per day was suitable for the production of <40 mg unfiltered BOD/l and <60 mg SS/l (95-percentile values), meeting the general requirements for discharge to surface waters. If the environmental regulator

specifies a maximum ammonia concentration for the final effluent of 10 mgN/l or less, then the rock filter should be aerated. For agricultural reuse a 2-3 log unit pathogen reduction is required for restricted irrigation and a 6-7 log unit pathogen reduction for unrestricted irrigation (WHO, 2006). The effluent contained <1000 faecal coliforms (FC) per 100 ml, and often <100 per 100 ml in summer, and was therefore suitable for restricted crop irrigation in summer, as the pathogen reduction, as indicated by this level of FC removal, would achieve the 2-3 log unit reduction required. In tropical climates, the authors recommended that an HLR of 0.5-1 m^3 of facultative pond effluent per m^3 of gross rock filter volume per day should be used. Assuming a wastewater flow of 0.07 m^3 per person per day, an HLR of 0.75.d^{-1} and a water depth in the rock filter (D_{rf}) of 0.6 m, the rock filter area is 0.16 m^2 per person. Thus the area of the anaerobic and facultative ponds and the rock filter would then be 0.76 m^2 per person (i.e. around 1 m^2 per person overall). Effluent quality could be estimated on the basis of 80 per cent BOD removal in the anaerobic and facultative ponds and 50 per cent in the rock filter; i.e. a cumulative BOD reduction of 90 per cent. In tropical climates anaerobic and facultative ponds and either unaerated rock filters or, if ammonia reduction is required, subsurface horizontal-flow or vertical-flow constructed wetland, can be used if the effluents are discharged to surface waters. If the treated wastewater is to be used for crop irrigation in tropical (or other warm) climates, then a 3-log unit pathogen reduction by treatment in a series comprising an anaerobic, a facultative and a single maturation pond is required for both restricted and unrestricted irrigation, provided that, in the case of unrestricted irrigation, there are in place post-treatment health-protection control measures that together provide a further 4-log unit pathogen reduction.

1.3 Combined WSP-duckweed systems

As described in the above section, WSP systems effectively remove bacterial pathogens from wastewater as a result of light, in combination with high pH values and high oxygen concentrations that accelerate the decay of bacterial pathogens. A drawback of shallow SP is the low efficiency of TSS and BOD removal, due to the presence of algae in the effluent. This could result in difficulties to satisfy discharge criteria for BOD or in reuse applications for drip-irrigation. Systems with a plug-flow hydraulic regime were found to be more efficient in pathogen removal However, conventional WSP cannot usually be designed as a plug-flow system because the excessive loading of the first pond(s) will cause anaerobiosis. Anaerobiosis is associated with odor generation and poor bacterial-pathogen removal.

Duckweed ponds (DP) effectively remove nutrients from the water, but an important sanitary disadvantage of DP is their poor performance with respect to bacterial pathogen removal due to the reduced light penetration into the water.

Post-treatment of effluent from an Upflow Anaerobic Sludge Blanket (UASB) reactor that was fed with domestic sewage, was conducted in an integrated pond system consisting of a series of shallow duckweed and stabilization ponds by Van der Steen et al. (1999). Although COD was reduced in the UASB, the effluent still contained high concentrations of faecal micro-organisms. The main objective of post-treatment was the removal of bacterial pathogens and further polishing of effluent quality. The authors aimed to show that rapid and efficient pathogen removal could be achieved in shallow stabilization ponds, and that passing the stabilization pond effluent through duckweed ponds was expected to remove algae due to reduced light penetration, leading to effluent with a high quality in respect to TSS and BOD. The pilot system consisted of 10 ponds in series, arranged in 3 stages, with a retention time of 4.2 days. The first stage consisted of 2 duckweed ponds, the second stage of 3 stabilization ponds and the third stage 5 duckweed ponds. The system's effluent median fecal coliform count in two experimental periods of 6 months was 3.3×10^2 - 5.0×10^3 per 100 ml. Increasing the retention time of the stabilization ponds to 3-4 days was suggested for consistently satisfying the WHO criterion for unlimited irrigation. Rapid removal took place in the stabilization ponds. A first order fecal coliform decay constant Kd was calculated for each of the three stages. The values obtained were 0.7-3.2, 4.0-5.9 and about $1.4d^{-1}$, respectively. The shading by the duckweed cover in the last stage proved to be able to remove practically all algae. Therefore, an excellent effluent quality with respect to TSS was achieved (11 mg/l). It was demonstrated that duckweed biomass-production and wastewater treatment for reuse in irrigation can be achieved in one simple system.

In the same pilot study described above, Van der Steen et al. (1998) investigated the nitrogen removal efficiency of anaerobically treated wastewater. Pond system influent nitrogen was mainly (90%) ammonium since organic nitrogen was hydrolysed in the UASB reactor. The nitrate concentration was only about 1.5 mg.l-1 NO_3-N, therefore most of the nitrogen available to the duckweed was in the form of ammonium. Optimal growth was therefore expected as duckweed preferentially utilise ammonium. Production of duckweed varied from 4.1-16.4 g/(m^2/day), and the highest production was achieved in the first pond, at 7.4-16.4 g/(m^2.day). The regression analysis suggested that this might be due to a higher concentration of dissolved organic compounds (COD-filtered) in the pond water of the first pond. The integrated pond system removed 50% of influent nitrogen. Volatilization and denitrification, duckweed growth, sedimentation and nitrification were responsible for approximately 73%, 18%, 6% and 3% of the ammonia removal respectively. Volatilization was therefore the major mechanism for nitrogen removal, and the pH in the ponds was very important.

As discussed previously, algal photosynthesis is crucial for efficient faecal coliform, (FC) decay, because it causes a rise in the pH and DO. However, algal matter may also have a negative effect on FC decay due to light attenuation. Van der Steen et al. (2000) therefore investigated whether suppressing algal development by inserting duckweed ponds in between a series of algal ponds could enhance the FC removal efficiency. The FC decay in a series of five shallow algal ponds receiving effluent from a UASB was compared to the decay in an integrated system of algal and duckweed ponds. The integrated system consisted of five mini-ponds (30 cm depth) in series: duckweed pond - algal pond - duckweed pond - algal pond - duckweed pond. The environmental factors that were known to affect FC decay i.e. sunlight radiation, pH and DO, were monitored and related to decay rates. In the algal ponds of the conventional system the light attenuation by algal matter became rate-limiting for the FC decay. In the integrated system, the algal concentration in the algal ponds was reduced by the intermediary duckweed ponds. This was shown to increase the FC decay in the algal ponds of the integrated system considerably, compared to the FC decay in the algal ponds of the conventional system. An improved system of duckweed and algal ponds was proposed, that was expected to reduce significantly the area requirements of pond systems.

1.4 Conclusion

Algal-based systems are dependent on a number of factors, of which available light is but one. Algae have a short doubling time; however, as cells multiply, their concentration increases, resulting in an increased turbidity. Less light penetrates and the growth of algae is limited. In this way, equilibrium is quickly established. Sufficient light is also required for bacterial destruction. Removal of faecal coliforms is effective in an algae-based WSP system. A disadvantage is that the algal cells remain in suspension and escape in the effluent. The presence of algae is indicated by a high COD and suspended solids concentration, often exceeding the general standards. This is one reason why WSP systems seldom comply.

Duckweed-based WSP systems have a distinctive floating mat of duckweed covering the surface of the ponds. It has been demonstrated that these systems are able to remove COD and nutrients effectively. Since they inhibit algal growth, the effluent is free from suspended material and therefore has a lower COD as compared with algae-based WSP systems. The disadvantage is that production of oxygen is limited to the surface layer associated with the mat of duckweed, and the water column remains essentially anaerobic. Higher life-forms such as protozoa and their predators can therefore not be established. The important mechanism of grazing on bacteria is absent, thereby reducing the efficiency of faecal coliform removal. There is also low penetration of sunlight. This explains why it appears that the ponds are under designed with respect to faecal coliform removal.

There is a worldwide trend to include a polishing step following algal-based ponds, particularly rock filtration system, often with aeration, to remove the suspended algal cells. It has been shown that when combining duckweed and algal-based systems following an upflow anaerobic sludge blanket reactor, the final effluent is of a better quality than when an algal-based system is employed alone.

Thorough studies have been conducted on the design parameters and modeling of algal-based WSP systems. However the knowledge base for the design of duckweed ponds is limited and the systems that have been modelled are often based on modified algal ponds that do not take the mechanism of duckweed growth and the necessity for harvesting into consideration.

1.5 Further research

Based on the findings of this literature review, and on our observations of existing pond systems, the following areas of further research have been identified, which will be addressed in this study:

- There is a need for more in-depth research into the identification and quantification of the mechanism and kinetics of duckweed growth and nutrient removal in duckweed ponds. We aim to model in detail the kinetics, hydrodynamics and mixing requirements of a duckweed-based system on a pilot scale level, in order to understand more about the necessary design parameters.
- As the duckweed systems that have been modeled in the literature were mostly based on modified algal pond systems, little research has been done into the ideal rates of duckweed harvesting, harvesting methods and overflow weir design. These parameters will be investigated and optimized where possible.
- There is little data available on the possible advantages of a combined duckweed/algal pond system in the absence of anaerobic digestion. These two systems will be combined in a pilot study with the aim of mitigating the disadvantages of each system by the advantages of the other.
- The use of rock filters as a polishing step for the final effluent of algal ponds has been shown to be successful. However, the literature shows that the effluent of a duckweed-based system has a low COD and suspended solids concentration. A rock filter system will be tested on a pilot scale if the final quality of the combined system requires further polishing. This may only be necessary in the winter months when there is expected to be reduced duckweed growth.
- It has been observed that micro-organisms such as viruses, cysts of parasites and *Vibrio cholerae* are removed by different mechanisms than faecal coliforms.

Dissolved hydrogen sulphide is toxic to *V. cholerae*. Hydrogen sulphide is formed under the anaerobic conditions in the facultative pond and underneath the duckweed cover. By maintaining a duckweed system in the facultative and second pond, it is hypothesized that the anaerobic conditions that will be maintained will also result in the effective destruction of *V. cholerae*. The study will also therefore aim to show that the use of duckweed in the initial ponds will increase the death rate of *V. cholerae* when compared to algal-based ponds.

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CHAPTER 2 EXPERIMENTAL WORK

2.1 Introduction

Conventional wastewater treatment systems are unlikely to be suitable for developing countries due to the lack of finance for construction and running costs and lack of skilled staff. Waste stabilisation ponds (WSP) are likely to be appropriate in hot climates, as they are low in cost and easy to operate and maintain. However they may not achieve secondary effluent standards in terms of reduction of TSS and nutrients because of algal growth in the ponds. Therefore duckweed-based wastewater treatment becomes more promising to achieve effluent standards including those for nutrients. In addition to reducing organics and pathogens, duckweed-based systems can also reduce concentrations of nutrients (ammonium and phosphates). Compared to other wastewater treatments, duckweed-based systems have several advantages such as high nutrient removal, inhibition of algal growth, prevention of odour and insect breeding through the development of a physical barrier, reduction of the effect of chlorine by-products, relatively low cost and high possibility for income generation, for example through the sale of composted duckweed for fertilizer, or as animal feed. However the duckweed mat results in low pathogen removal, and an inability to receive shock loading if duckweed-based water treatment is used without other treatment methods (Smith & Moelyowati, 2001).

Thorough studies have been conducted on the design parameters and modeling of algal-based WSP systems. However the knowledge base for the design of duckweed ponds is limited and the systems that have been modeled are often based on modified algal ponds that do not take the mechanism of duckweed growth and the necessity for harvesting into consideration.

Based on the findings of the literature review, and on observations of existing pond systems, the following areas of further research were identified, which will be addressed in this study:

- There is a need for more in-depth research into the identification and quantification of the mechanism and kinetics of duckweed growth and nutrient removal in duckweed ponds. We aim to model in detail the kinetics, hydrodynamics and mixing requirements of a duckweed-based system on a pilot scale level, in order to understand more about the necessary design parameters.
- As the duckweed systems that have been modeled in the literature were mostly based on modified algal pond systems, little research has been done into the ideal

rates of duckweed harvesting, harvesting methods and overflow weir design. These parameters will be investigated and optimized where possible.

- There is little data available on the possible advantages of a combined duckweed/algal pond system in the absence of anaerobic digestion. These two systems will be combined in a pilot study with the aim of mitigating the disadvantages of each system by the advantages of the other.
- The use of rock filters as a polishing step for the final effluent of algal ponds has been shown to be successful. However, the literature shows that the effluent of a duckweed-based system has a low COD and suspended solids concentration. A rock filter system will be tested on a pilot scale if the final quality of the combined system requires further polishing. This may only be necessary in the winter months when there is expected to be reduced duckweed growth.

2.2 Materials and methods

2.2.1 Duckweed stock culture

Duckweed was collected from a dam receiving effluent water from a waste water treatment plant in the area of Cullinan, Gauteng. The initial culture was a mixed culture consisting of *Lemna turionifera*, *Wolffia* spp. and *Lemna gibba*. The duckweed was washed in tap water to remove any debris, but was not sterilized. A stock culture of duckweed was maintained at 25°C for experimental work.

2.2.2 Reactor experiments

2.2.2.1 Controlled temperature and light intensity

In order to determine the effects of temperature, nutrient concentration and harvesting rate on the growth rate of the duckweed culture, three baffled plug flow reactors with recirculation were set up in temperature controlled rooms, at 25°C, 18°C and 13°C respectively. The light intensity, pH and nutrient concentrations for each chamber were kept constant, and an artificial nutrient solution was used instead of sewage effluent to eliminate inconsistent conditions, contamination and health risks to laboratory staff.

Each reactor was approximately 270L, and was divided into three sections, each with its own small recirculation pump. The volume of each section was approximately 90L, and was divided on the surface into four separate chambers of approximately equal surface area. Five vertical baffles were installed into each section to ensure adequate mixing and to reduce surface turbulence. A schematic side view drawing of a single reactor section showing the baffle set up and re-circulation is presented in Figure 2-2. A tracer study was conducted in

order to determine the recycle rate and dispersion in the reactors using fluorescence dye as a qualitative marker and NaCl. The purpose of the tracer study was to ensure that the supply of nutrients to the duckweed was not diffusion limited. The conductivity was tracked with a conductivity meter.

The tanks-in-series model has been used to model the dispersion in the reactor. The reactor was modeled as a closed recirculation system and the procedure described by Levenspiel (1999) was followed. The tracer was introduced as a pulse in the suction of the recirculation pump and the conductivity was measured just upstream of the same point. The normalized tracer signal was plotted in Figure 2-1 and simulated with equation 2.1.

$$C_{,pulse} = Ne^{-N} \sum_{m=1}^{\infty} \frac{(N\ )^{mN-1}}{(mN-1)!} \qquad (2.1)$$

where N is the number of tanks-in-series,

m is the number of passes and

Θ is the dimensionless time based on the residence time, τ, in all N tanks,

where Θ =t/τ.

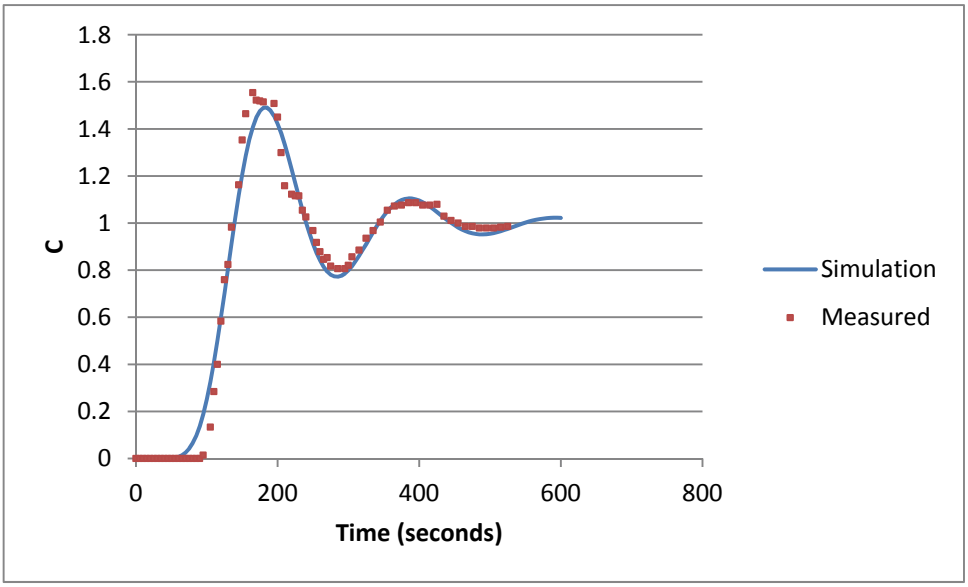

Figure 2-1: Tracer signal in the recirculation system

With a reactor volume of 90 L and a flow rate of 0.46 L/s, the C-curve could be simulated with 13 tanks-in-series. Based on this information, it was concluded that any disturbance will be attenuated within less than 2.2% of the mean value after 3 passes of the recycle where t = 3Θ or 591 seconds. Considering that the time-scale of harvesting and replacement of

nutrients are orders of magnitude higher, one can safely assume that the transfer of nutrients will be sufficiently high to prevent diffusion limiting conditions.

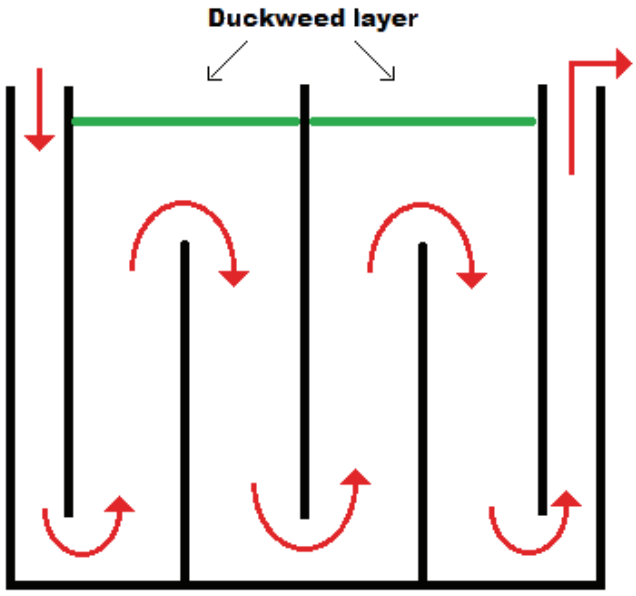

Figure 2-2: Schematic side view drawing of reactor, showing baffle design

The reactors were filled with tap water to 5 cm below the top, and two grow lamp tubes (Osram L36W/77 Floura lamps), were suspended 10 cm from the water surface of each of the three sections. This light set up is explained in more detail in section 2.2.2.1.1 below. The lights were set to turn on and off according to a 16h light cycle. Figure 2-3 and Figure 2-4 show the reactor set up, including the recirculation pumps and chamber divisions.

Figure 2-3: Reactor and light set up (left) and recirculation pumps (right)

Figure 2-4: Growth chamber divisions

Each chamber of each reactor was monitored daily for dissolved oxygen concentration using a DO 6+ Dissolved Oxygen Meter (Eutech Instruments) both at the surface and at the bottom of the chamber before the lights came on in the morning, and after they had been on for at least 8h. pH was measured once daily with a Cyberscan 510 pH meter in each reactor and was adjusted to between 7 and 8 pH units when necessary with 1 M solutions of HCl or H_2SO_4. The water level was maintained by adding tap water on a daily basis to account for evaporation.

Each of the chambers of were inoculated with a similar mass of duckweed stock culture to ensure a uniform coverage of the chamber surface, and the duckweed was allowed a week to acclimatize to the reactor conditions before harvesting of the culture was commenced.

2.2.2.1.1 Light intensity requirements

Plants convert radiant energy into chemical energy between 400 and 700 nanometers (nm), the region known as photosynthetically active radiation (PAR). Photosynthetic activity is at a peak through the absorption of radiant energy between 400 and 510nm (blue light) and again between 610 to 700nm (red light). Light at wavelengths between 510 and 610nm (green-yellow light) has little effect on plant growth. The energy of a photon is inversely proportional to its wavelength:

$$E=hv=\frac{hc}{\lambda} \qquad (2.2)$$

where E=the energy of a photon or quantum of radiant energy,

h=Planck's constant ($6.626x10^{-34}$ J.s)

c=speed of light ($2.998X10^{8}$ m/s)

λ=wavelength (m)

Illumination for plants, also known as irradiance, is sometimes measured in PAR watts per square meter ($W.m^{-2}$). Another means of measuring light quantity for plant growth involves discrete units of quantum flux in the PAR region called photons. Photon flux is commonly measured in units of micromoles per square meter per second ($\mu moles.m^{-2}.s^{-1}$), where 1 mole of photons equals 6.022×10^{23} photons. This was formerly referred to as µEinsteins per square meter and per second, or $\mu E.m^{-2}.s^{-1}$

This is an objective measure since it directly indicates how much light energy is available for plants to use in photosynthesis. However, lamp manufacturers typically rate their lamps in lumens, a measure of light in the spectrum visible to humans, which includes green-yellow light. Moreover, lighting levels are measured in lumens per square meter (lux) or per square foot (foot-candles). Since the spectral sensitivities of plants and humans are quite different, there is no direct method of converting the units without evaluating the full range of spectral characteristics for a given light source. This conversion factor is normally supplied by lamp manufacturers.

Blackman & Robertson-Cunninghame (1954) tested the response of a culture of *Lemna minor* to three different light intensities, namely 180, 275 and 700 foot candles. At 25°C, cultures exposed to a light intensity of 700 foot candles took 2.23 days to double their weight when compared with 4.14 days for cultures exposed to intensity of 180 foot candles. The conversion factor of foot candles to lux is approximately 1:10.764. The range of light intensities tested by Blackman & Robertson-Cunninghame were therefore 1937.52 lux-7534.8 lux.

Lasfar et al. (2007) supplied their *Lemna minor* cultures with a light intensity of 371 $\mu mol.m^{-2}.s^{-1}$, which was in excess of the light saturation point of *Lemna minor* of 342 $\mu mol.m^{-2}.s^{-1}$. This value was unfortunately not cited by the authors.

Zayed et al. (1998) studied the phytoaccumulation of duckweed. All cultures were maintained in growth chambers at 25°C and at an irradiance of 400 PFD $m^{-2}.s^{-1}$ supplied over a 16 hour day length.

Wedge & Burris (1982) found that *Lemna minor* cultures were photo inhibited at light intensities greater than 1200 $\mu E.m^{-2}.s^{-1}$, and that at temperatures ranging from 15-30°C light saturation of photosynthetic O_2 evolution of *Lemna* occurred from 300-600 $\mu E\ m^{-2}.s^{-1}$.

Osram L36W/77 Floura lamps, which have a 36W capacity and emit light predominantly in the red and blue spectrum of light, as illustrated in Figure 2-5 below, were used in this study. According to the manufacturer, the conversion factor from µmol.m^{-2}.s^{-1} to lux is 34.48 in the 400-700nm light range.

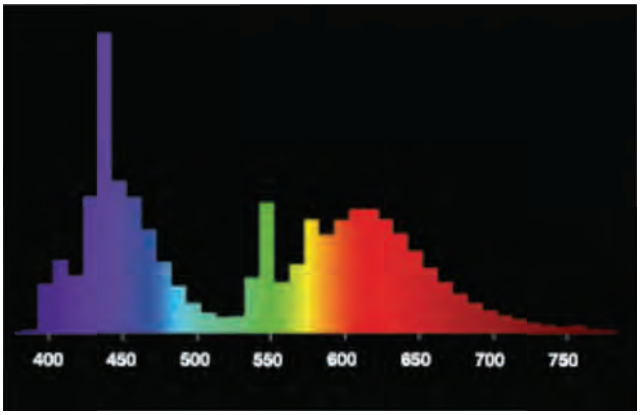

Figure 2-5: Light spectrum of the Osram 77 Floura lamps (nm)

The effective light intensity of the installed lamps was measured at 10 cm intervals from the source for three lights using a Major Tech MT940 light meter, the results of which are presented in Table 2-1.

Table 2-1: Effective light intensity (lux) of three lamps measured at 10 cm intervals from the source

Distance (cm)	Lamp 1 (lux)	Lamp 2 (lux)	Lamp 3 (lux)	Ave Lux	1/ Ave lux
0	13050	14050	13060	13386.67	7.47012E-05
10	4000	4050	3800	3950.00	0.000253165
20	2450	2410	2260	2373.33	0.000421348
30	1860	1929	1674	1821.00	0.000549149
40	1500	1683	1424	1535.67	0.000651183
50	1515	1468	1375	1452.67	0.000688389
60	1000	1420	1341	1253.67	0.00079766

The conversion of light intensity units from lux to µmol.m^{-2}.s^{-1} for the average light intensity data is presented in Table 2-2.

Table 2-2: Conversion of average measured light intensity values from lux units to µmol.m^{-2}.s^{-1}

Distance (cm)	Ave Lux	Ave µmol.m^{-2}.s^{-1}	1/Ave µmol.m-2.s-1
0	13386.67	388.21	0.002575903
10	3950.00	114.55	0.008729812
20	2373.33	68.83	0.014529252
30	1821.00	52.81	0.018936166
40	1535.67	44.53	0.022454586
50	1452.67	42.13	0.023737558
60	1253.67	36.36	0.027505524

Based on the conversion factor of 0.029, the average light intensity at the source was 388.2 µmol.m^{-2}.s^{-1}, and at 60 cm, the average light intensity was 36.35 µmol.m^{-2}.s^{-1} (Table 2-2). When comparing these values with the values reported in literature (Zayed et al., 1998, Wedge & Burris, 1982, Lasfar et al, 2007), the effective light intensity at 60 cm was in the order of 10 times lower than the saturation light intensity for *Lemna* cultures. At 10 cm from the source the effective intensity is an average of 114 µmol.m^{-2}.s^{-1}. When comparing the measured values to those reported by Blackman & Robertson-Cunninghame (1954), the optimum light intensity of 7534.8 lux was evident between 0 and 10 cm from the source.

Using the formula of the straight line graph of the inverse of the light intensity versus the distance from the source, the distance required from the light source to provide the optimum intensity of approximately 7534.8 lux could be calculated to be 3.27 cm

Therefore in order to supply the duckweed culture with the light intensity described by Blackman & Robertson-Cunninghame (1954), the light will need to be 3.27 cm or away from the culture.

Similarly, the distance from the light source required to supply the saturation light intensity of 342 µmol.m^{-2}.s^{-1} as recommended by Lasfar (2007) can be calculated, to give a distance from source of approximately 2.3 cm.

At these small distances only the duckweed directly below the light source would be subject to light of the required intensity; those situated obliquely would likely receive a lower intensity. It was therefore decided that the height of the lamps should be adjusted to 10 cm from the culture, which will provide an average light intensity of 3950 lux, or 114 µmol.m^{-2}.s^{-1} to the duckweed culture. This is approximately one third of the

recommended optimum light intensity. It is therefore expected that the cultures may be sensitive to variation in light intensity.

2.2.2.1.2 Nutrient concentration

Each 90L section of the reactors was spiked with a different nutrient concentration, based on varying dilutions of the Huttner media (Vermaat & Hanif, 1998). Five dilutions were tested, namely 1/5, 1/25, 1/100, 1/150 and 1/200, in order to determine the limiting nutrient concentrations. The composition of the media and dilutions applied are presented in Table 2-3, and the respective ammonium nitrogen, nitrate nitrogen and phosphorus concentrations of each solution are presented in Table 2-4.

Table 2-3: Dilutions of Huttner media applied

Nutrient Compound	Concentration (mg/L)					
	Undiluted Huttner media	**1/5**	**1/25**	**1/100**	**1/150**	**1/200**
NH_4NO_3	200	40	8	2	1.333	1
KH_2PO_4	312.5	62.5	12.5	3.125	2.083	1.5625
$Ca(NO_3)_2.4H_2O$	200	40	8	2	1.333	1
$MgSO_4.7H_2O$	500	100	20	5	3.333	2.5
$FeCl_3.6H_2O$	25	5	1	0.25	0.167	0.125
Na_2-EDTA	500	100	20	5	3.333	2.5

Table 2-4: Nutrient composition of each solution

Nutrient Compound	Concentration (mg/L)				
	1/5	**1/25**	**1/100**	**1/150**	**1/200**
NH_4-N	7	1.4	0.35	0.23	0.175
NO_3-N	9.5	1.86	0.462	0.31	0.2325
PO_4-P	14.26	2.852	0.713	0.475	0.3565

The nutrient media in the reactors was replaced once a week to maintain a reasonably constant concentration of nutrients.

Because the duckweed culture was not axenic, no source of COD was added to the solution.

2.2.2.1.3 Harvesting regime

Each of the four chambers at each nutrient concentration was harvested at a different rate, by removing a specific percentage of the duckweed surface area. By harvesting this percentage of the surface three times a week, the culture ages were maintained at either 12d, 23d, 35d, 47d or 58d. The culture ages maintained in the 25°C reactor were 12d, 23d, 35d and 47d for all nutrient concentrations tested, but the reactors at 18°C and 13°C were harvested to maintain longer culture ages at nutrient concentrations of 1/5 and 1/25. The 12d culture was eliminated and was substituted with a 58d culture, because of the early washout of duckweed experienced at a 12d culture age at these temperatures.

The wet mass of the harvested duckweed was determined, and the harvested material was then dried for 1h at 105°C to determine the dry mass.

Once the chambers appeared to have reached a steady state, the chemical oxygen demand (COD) and total phosphorus (TP) concentration per gram of the dried harvested plant material combined from each nutrient concentration at a specific temperature, was determined. A known mass of dry duckweed was homogenized in distilled water, and nutrient concentrations were determined using Hach TNT test kits, according to the manufacturer's instructions. Samples were digested using a Hach DRB 200 Dry Thermostat Reactor and samples measured with a Hach DR 3900 Spectrophotometer. Samples were sent to an accredited laboratory for the determination of the total Kjeldahl nitrogen (TKN) concentration.

2.2.2.2 Natural light and uncontrolled temperature

In the controlled temperature and light intensity experiments described above, variable conditions of nutrient concentration, temperature and culture ages were considered, but the light intensity was kept constant with artificial light in temperature controlled environments, with the goal of determining the effects of limiting conditions so as to provide data for a conservative full scale conceptual design. There are indications that the light intensity may pay an important role in the growth rate and effect of inhibiting nutrient concentrations. The maximum output of the artificial lights was less than 40% of that of natural sunlight, and it was therefore important that certain tests be repeated under natural light conditions to compare the findings under the light limiting conditions, and obtain more realistic design limits. Lasfar et al. (2007) reported a light saturation point of *Lemna minor* of 342 $\mu mol.m^{-2}.s^{-1}$. The conversion factor from $\mu mol.m^{-2}.s^{-1}$ to lux for sunlight is approximately 54, so this equates to approximately 18468lux. Above this light intensity there is not expected to be any additional effect on the growth potential of the culture.

Two reactors of the same design as described above were set up outside in natural light; one in full sun and the other undercover to give full-shade conditions. A third reactor was set up in the 25°C temperature controlled room as a control. The three main chambers of each reactor were filled with 1/5, 1/25 and 1/100 dilutions of Huttner media as described above. Each of the four chambers at each nutrient concentration was harvested at a different rate, as described previously, by removing a specific percentage of the duckweed surface area. The culture ages were maintained at either 7d, 12d 23d or 35d. A greater harvesting rate was tested here (7d culture age) than under controlled conditions as it was assumed that the growth rate of the duckweed would be greater under natural sunlight conditions. The wet and dry mass of the harvested duckweed was determined as described previously. The temperature and pH and dissolved oxygen concentration of each chamber was measured daily as described above, and the light intensity was measured three times per day at 10:00am, 12:00pm and 3:00pm.

As in previous experiments, the nutrients were replaced weekly to maintain a reasonably constant concentration of nutrients.

Once the chambers appeared to have reached a steady state, the chemical oxygen demand (COD) and total phosphorus (TP) concentration per gram of the dried harvested plant material combined from each nutrient concentration in the sun and shade was determined as described above. Samples were sent to an accredited laboratory for the determination of the total Kjeldahl nitrogen (TKN) concentration.

2.2.3 Nutrient uptake tests

2.2.3.1 Controlled temperature and light intensity

In order to monitor the rate of nutrient uptake from solution by the duckweed, 12 1L containers were set up in each temperature controlled room, under the same conditions of light intensity as the reactors. The containers were spiked with nutrients in groups of four, at 1/5, 1/25, and 1/100 Huttner media respectively. The containers were inoculated with a uniform surface layer of duckweed, and allowed to acclimatize for 1 week. The nutrient solutions were then changed, and the duckweed was harvested at the same rates as described for the reactors, namely 12d, 23d, 35d and 47d culture ages at 25°C, and 23d, 35d, 47d and 58d culture ages at 18°C and 13°C. pH, dissolved oxygen and conductivity (Orion 4 Star pH/conductivity meter from Thermo Electron Corporation) were monitored in the containers on a daily basis.

The concentrations of nitrate, ammonia and ortho-phosphorus were monitored in the 1/25 and 1/100 containers at 25°C, as these were shown in the reactor experiments to be the most optimal of the tested conditions for duckweed growth, in order to determine the kinetics of nutrient removal.

2.2.3.2 Natural light and uncontrolled temperature

In order to determine the effect of increased light intensity on the nutrient uptake of the duckweed, 9 1L containers were set up, 4 in the shade and 5 in the sun. It was observed in the temperature controlled nutrient uptake tests that the ammonia was depleted rapidly from solution at the concentrations tested. In order to observe this phenomenon more clearly, the nutrient media was modified for these tests by increasing the ammonia concentration so that the NH_4-N and NO_3-N concentrations were the same. $Ca(NO_3)_2.4H_2O$ was excluded from the solution. Two concentrations were tested; the higher concentration was tested in the sun and the shade, and the lower only in the sun, in 1 container only, in order to observe the effect of the initial starting concentration on the rate of nutrient uptake by the duckweed. The composition of the media used is presented in Table 2-5.

Table 2-5: Nutrient composition of the modified Huttner media

Nutrient Compound	**Concentration (mg/L)**	
	High	**Low**
NH_4-N	20	10
NO_3-N	20	10
PO_4-P	10	7

The four containers in the sun and shade at the higher concentration were harvested to maintain culture ages of 7d, 12d, 23d and 35d respectively. The container with the low concentration of nutrients was harvested to maintain a 23d culture age.

2.3 Results

2.3.1 Reactor experiments

2.3.1.1 Effect of nutrient composition and harvesting regime

2.3.1.1.1 Growth rate at 25°C

The dry mass surface densities for different dilutions of Huttner media for the 25°C reactor are presented in Figure 2-6, Figure 2-8, Figure 2-10, Figure 2-12 and Figure 2-14 and the dry masses of duckweed harvested in order to maintain the culture ages as indicated are presented in Figure 2-7, Figure 2-9, Figure 2-11, Figure 2-13 and Figure 2-15. Photographs of the surface areas of each chamber over time for each concentration at 25°C are presented in Table A-1, Table A-2, Table A-3, Table A-4 and Table A-5 in Appendix A.

Moving average trendlines have been used in the figures to illustrate the changes in surface density with time. Using linear trendlines, it was determined whether there was a net increase, decrease or a stable dry mass surface area in the chambers at different nutrient concentrations and harvesting rates. A summary of the results is presented in Table 2-6.

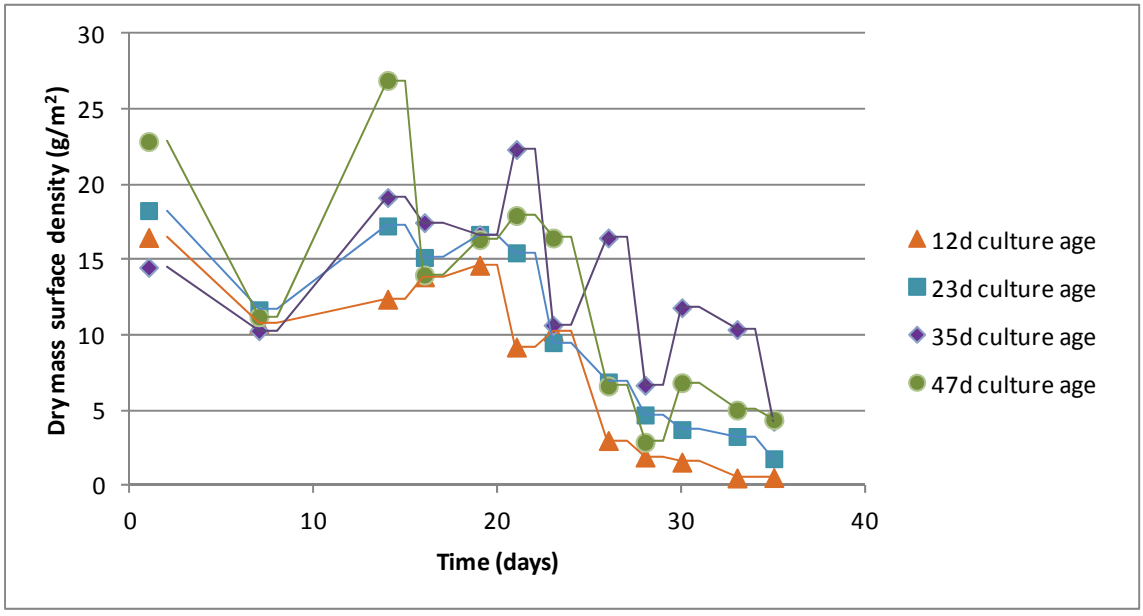

Figure 2-6: Dry mass surface density of different duckweed culture ages grown at 25°C in a 1/5 Huttner solution

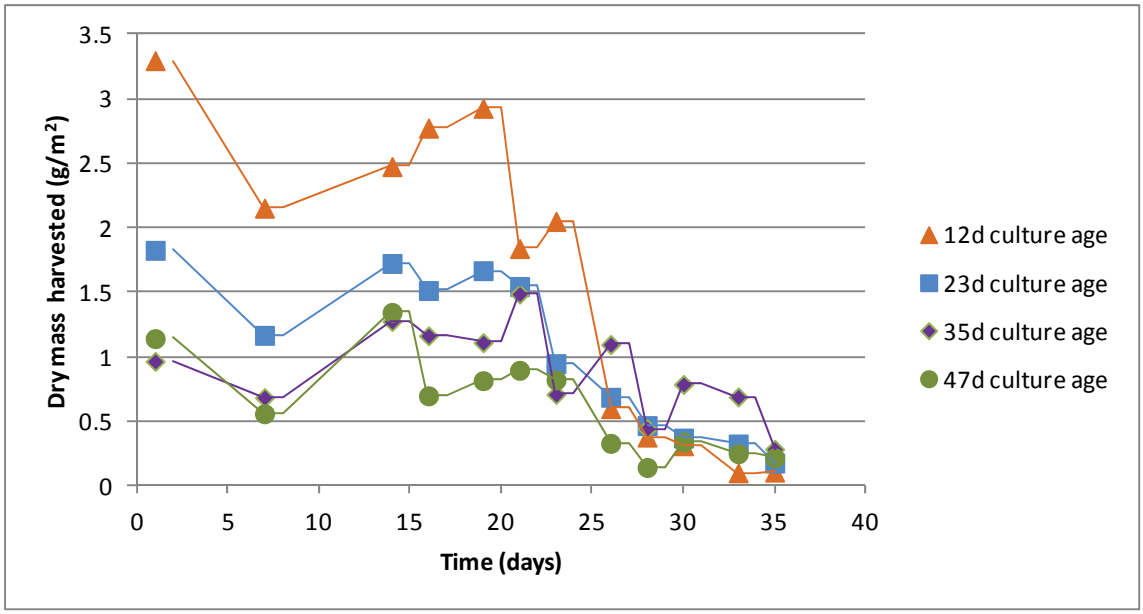

Figure 2-7: Dry mass of duckweed harvested to maintain culture ages at 25°C in a 1/5 Huttner solution

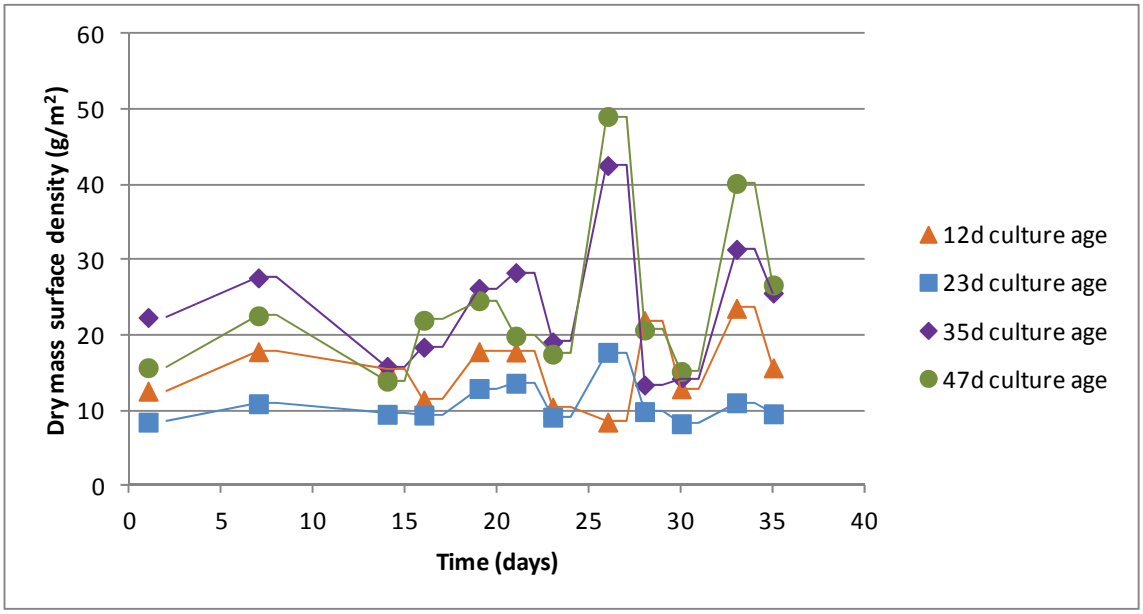

Figure 2-8: Dry mass surface density of different duckweed culture ages grown at 25°C in a 1/25 Huttner solution

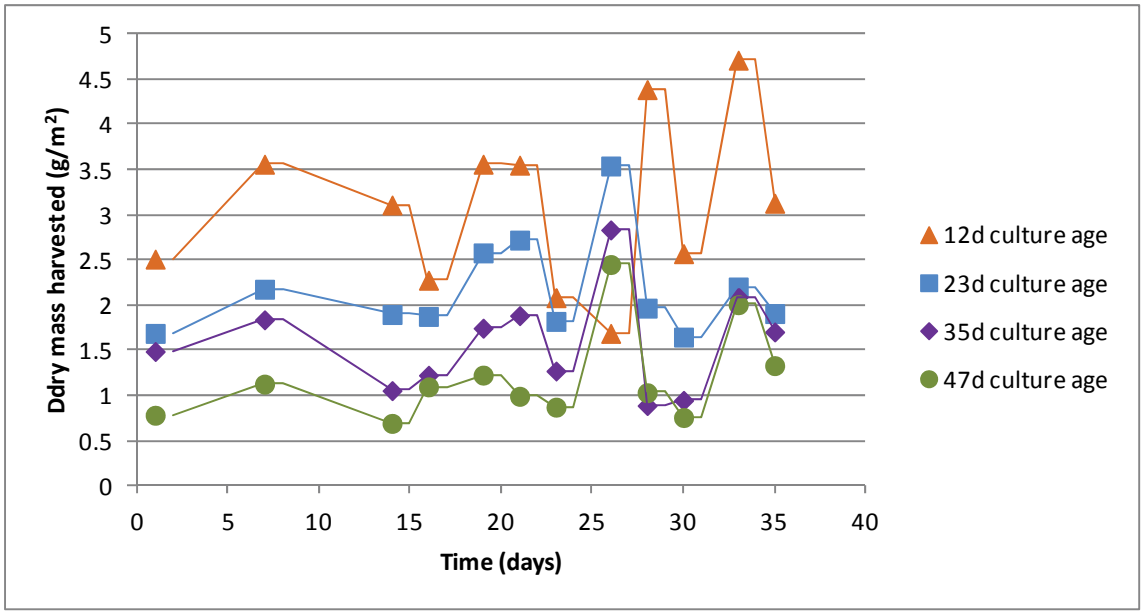

Figure 2-9: Dry mass of duckweed harvested to maintain culture ages at 25°C in a 1/25 Huttner solution

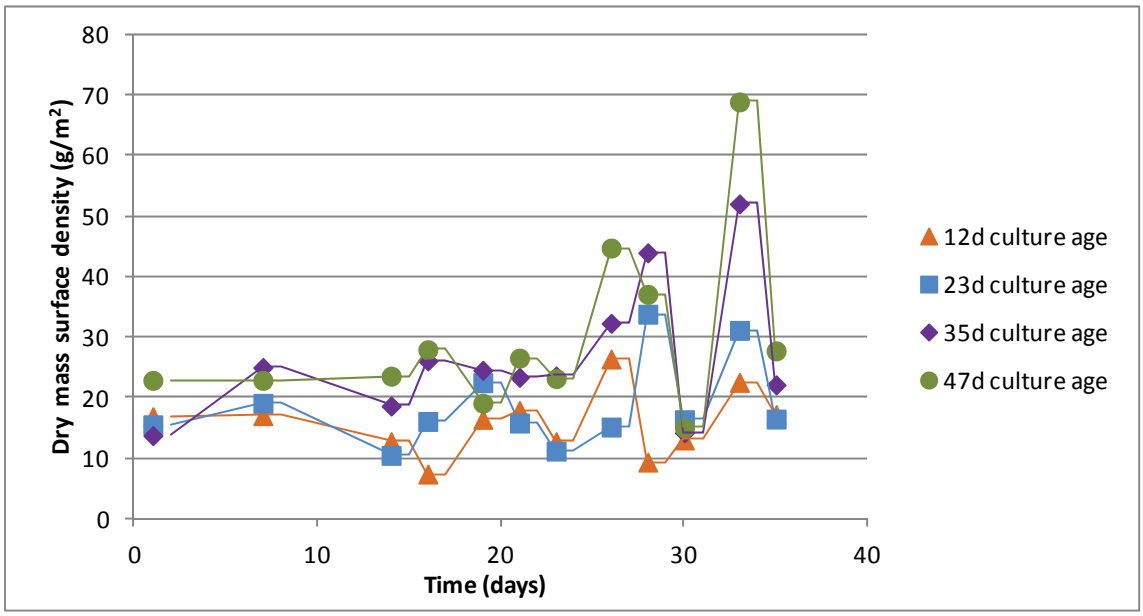

Figure 2-10: Dry mass surface density of different duckweed culture ages grown at 25°C in a 1/100 Huttner solution

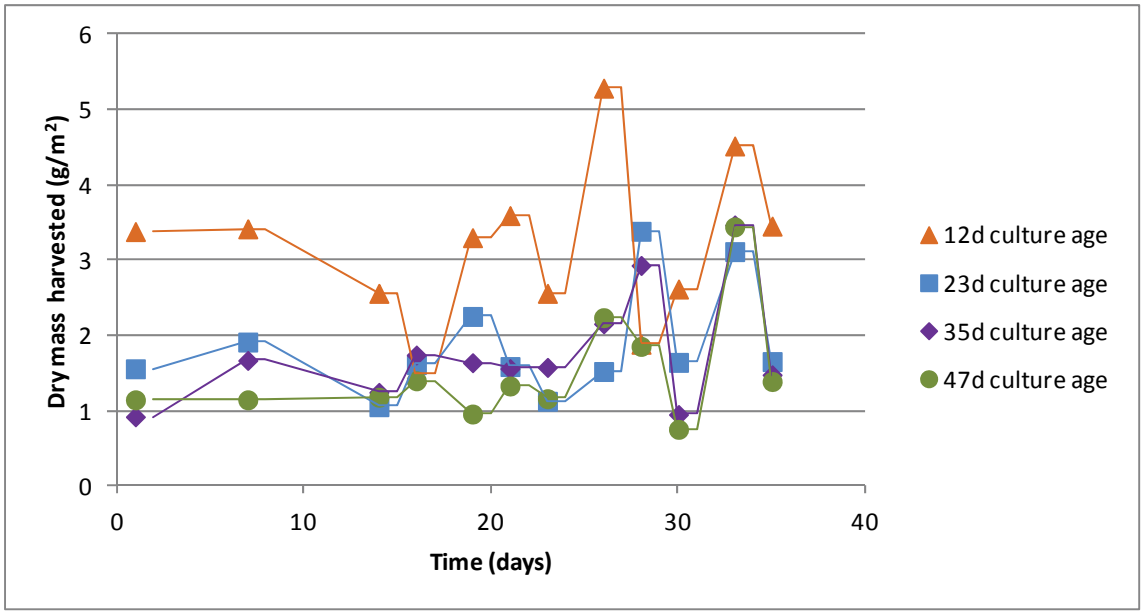

Figure 2-11: Dry mass of duckweed harvested to maintain culture ages at 25°C in a 1/100 Huttner solution

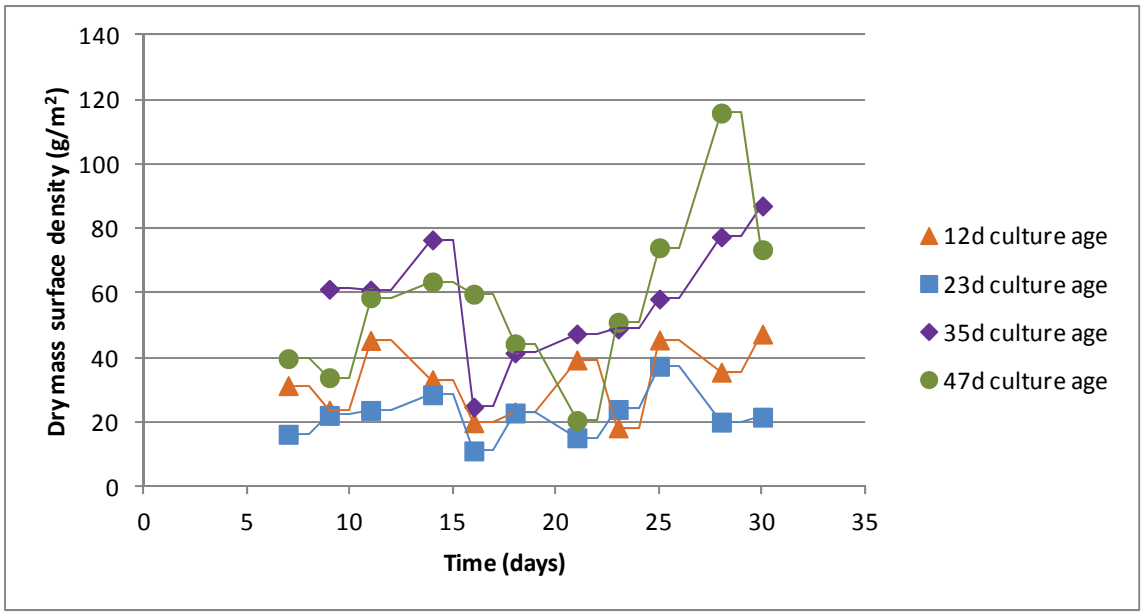

Figure 2-12: Dry mass surface density of different duckweed culture ages grown at 25°C in a 1/150 Huttner solution

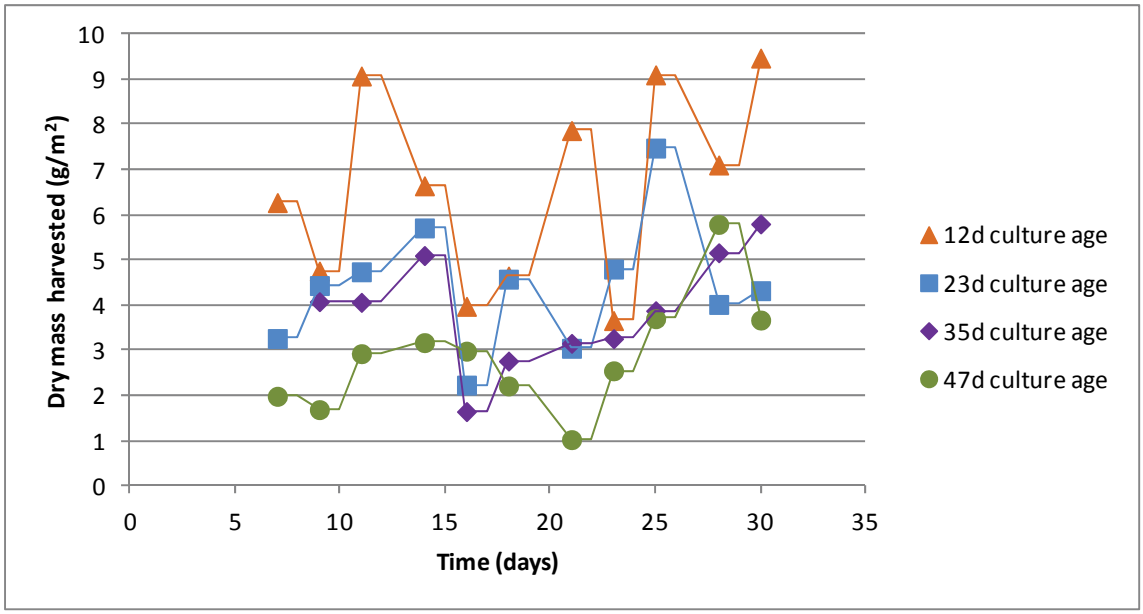

Figure 2-13: Dry mass of duckweed harvested to maintain culture ages at 25°C in a 1/150 Huttner solution

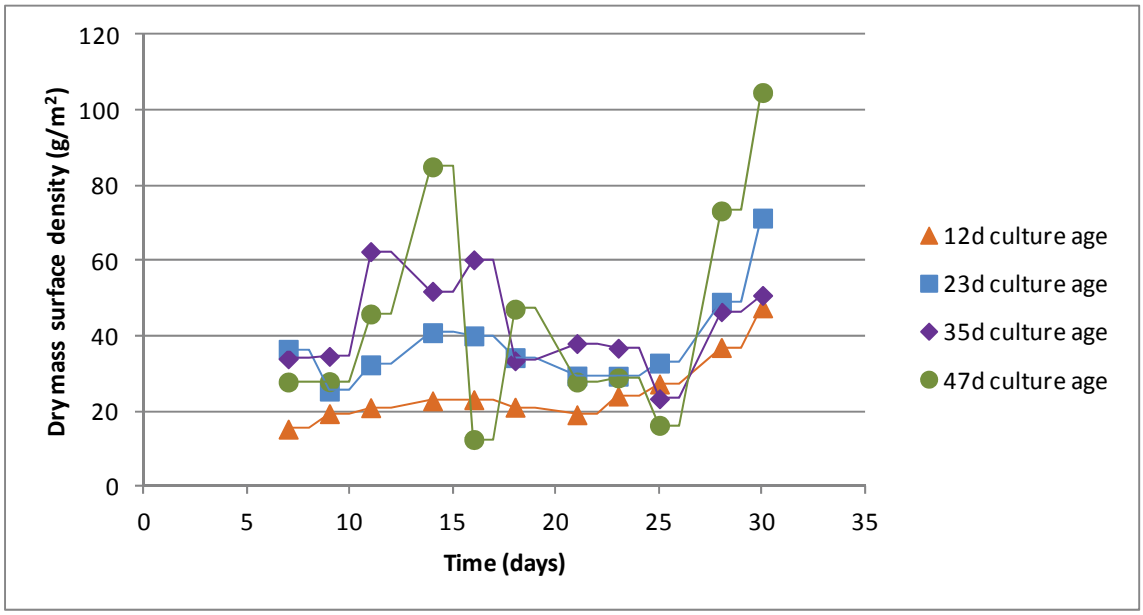

Figure 2-14: Dry mass surface density of different duckweed culture ages grown at 25°C in a 1/200 Huttner solution

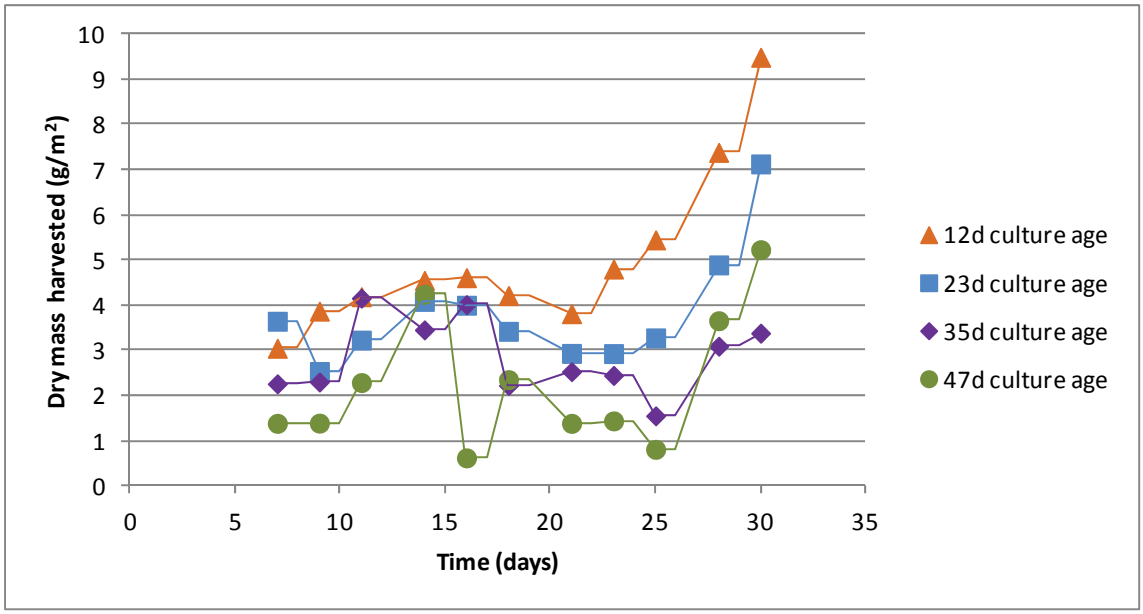

Figure 2-15: Dry mass of duckweed harvested to maintain culture ages at 25°C in a 1/200 Huttner solution

2.3.1.1.2 Growth rate at 18°C

The dry mass surface densities for different dilutions of Huttner media for the 18°C reactor are presented in Figure 2-16, Figure 2-18, Figure 2-20, Figure 2-22 and Figure 2-24, and the dry masses of duckweed harvested in order to maintain the culture ages as indicated are presented in Figure 2-17, Figure 2-19, Figure 2-21, Figure 2-23 and Figure 2-25. Photographs of the surface areas of each chamber over time for each concentration at 25°C are presented Table A-6, Table A-7, Table A-8, Table A-9 and Table A-10 in Appendix A. A summary of the results is presented in Table 2-6.

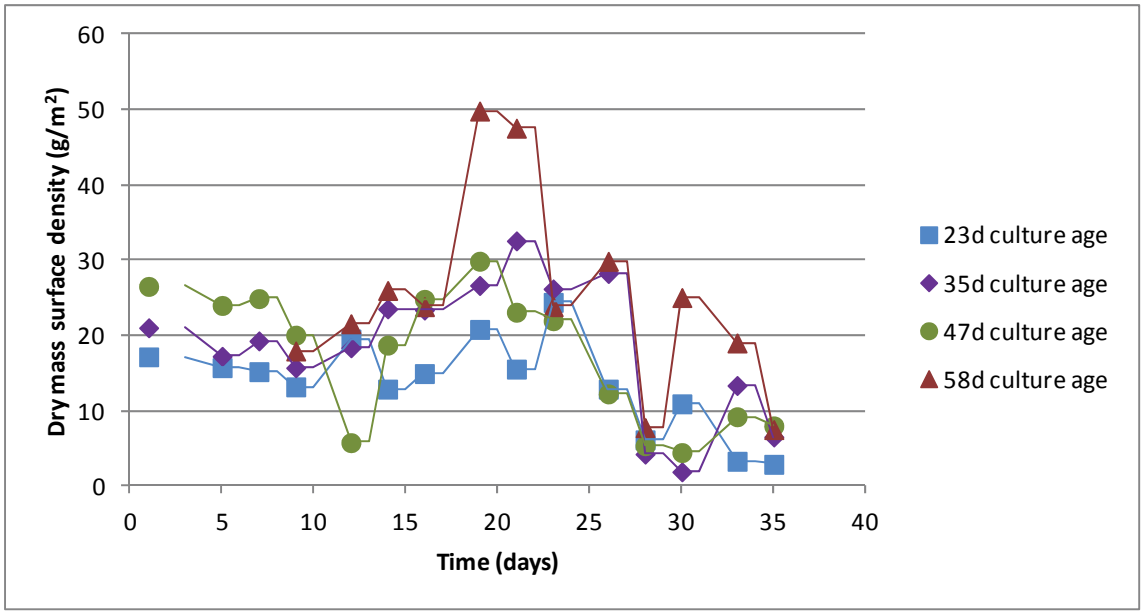

Figure 2-16: Dry mass surface density of different duckweed culture ages grown at 18°C in a 1/5 Huttner solution

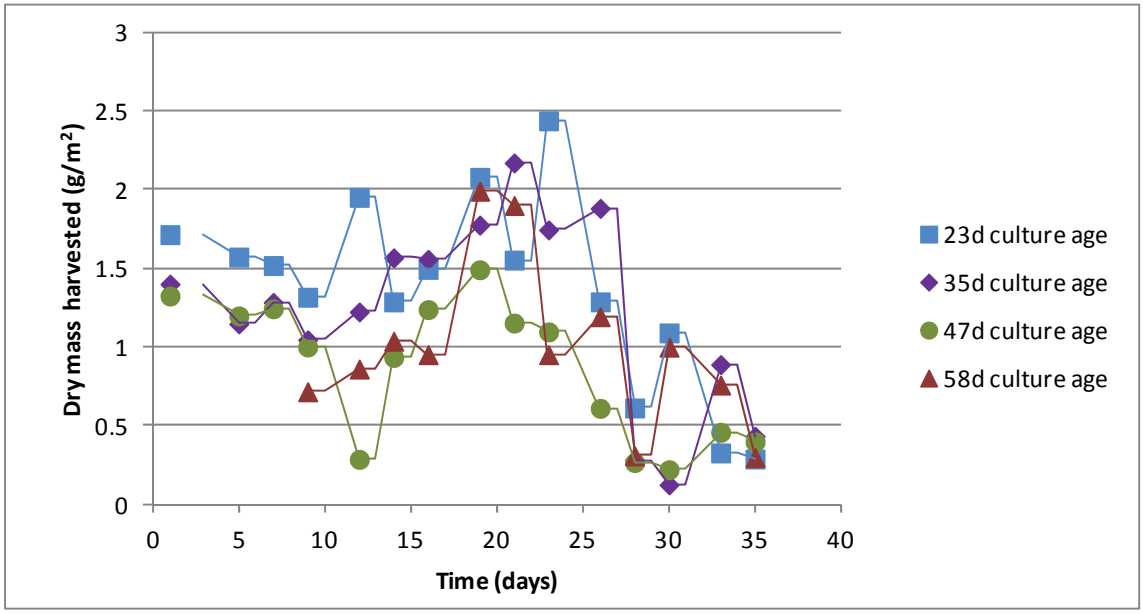

Figure 2-17: Dry mass of duckweed harvested to maintain culture ages at 18°C in a 1/5 Huttner solution

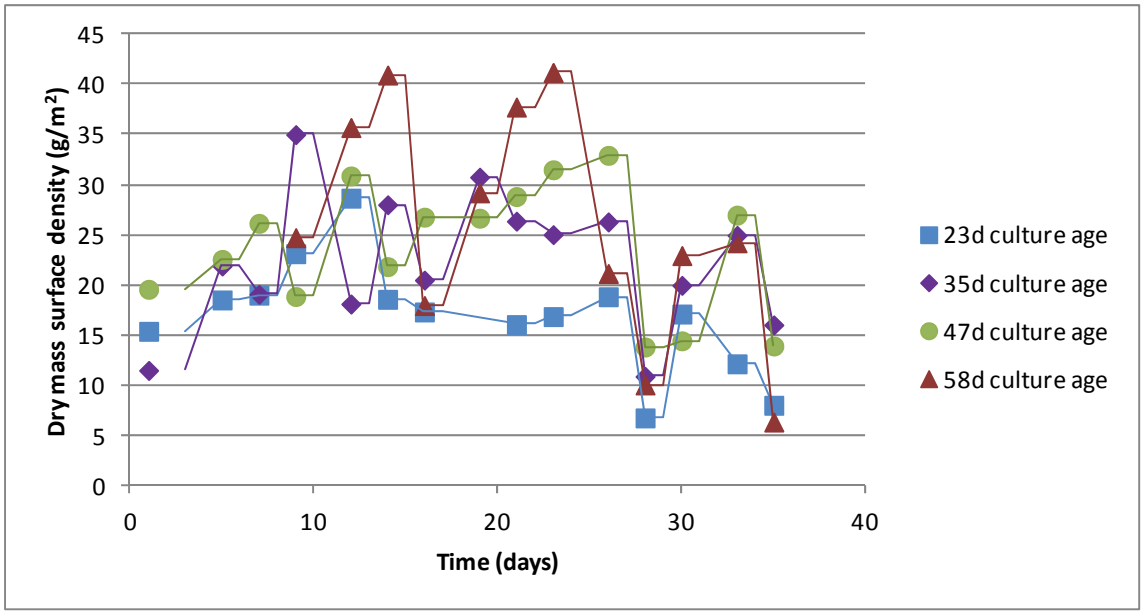

Figure 2-18: Dry mass surface density of different duckweed culture ages grown at 18°C in a 1/25 Huttner solution

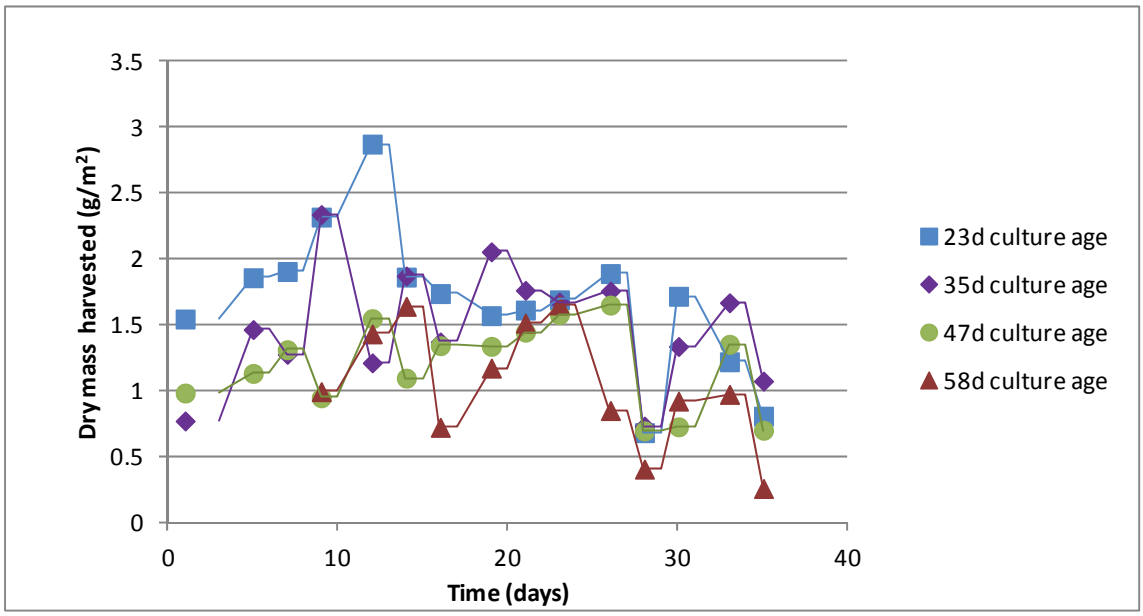

Figure 2-19: Dry mass of duckweed harvested to maintain culture ages at 18°C in a 1/25 Huttner solution

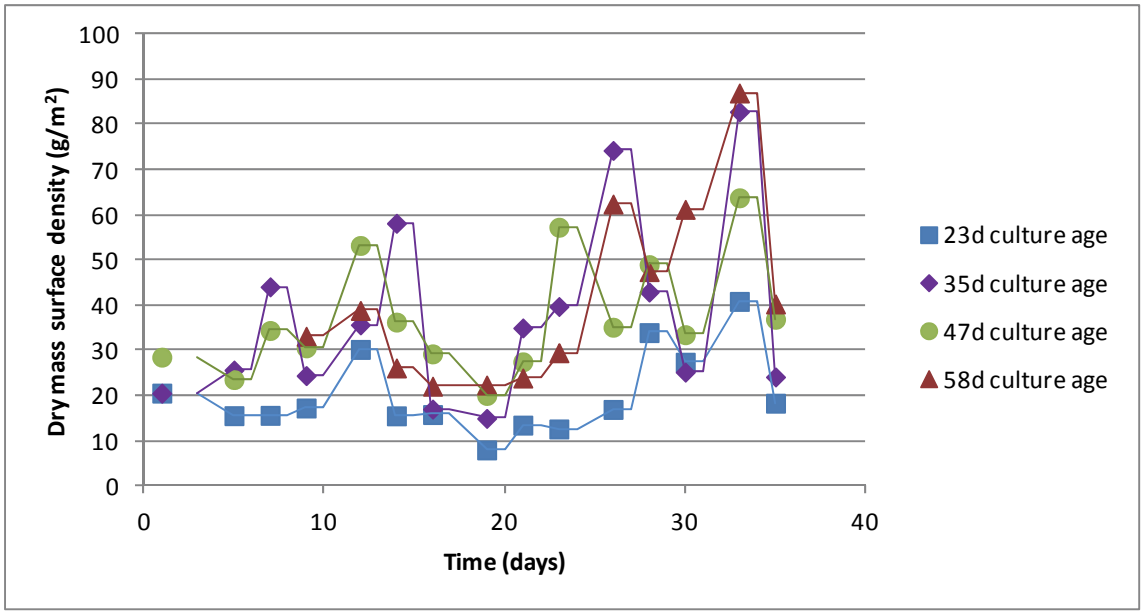

Figure 2-20: Dry mass surface density of different duckweed culture ages grown at 18°C in a 1/100 Huttner solution

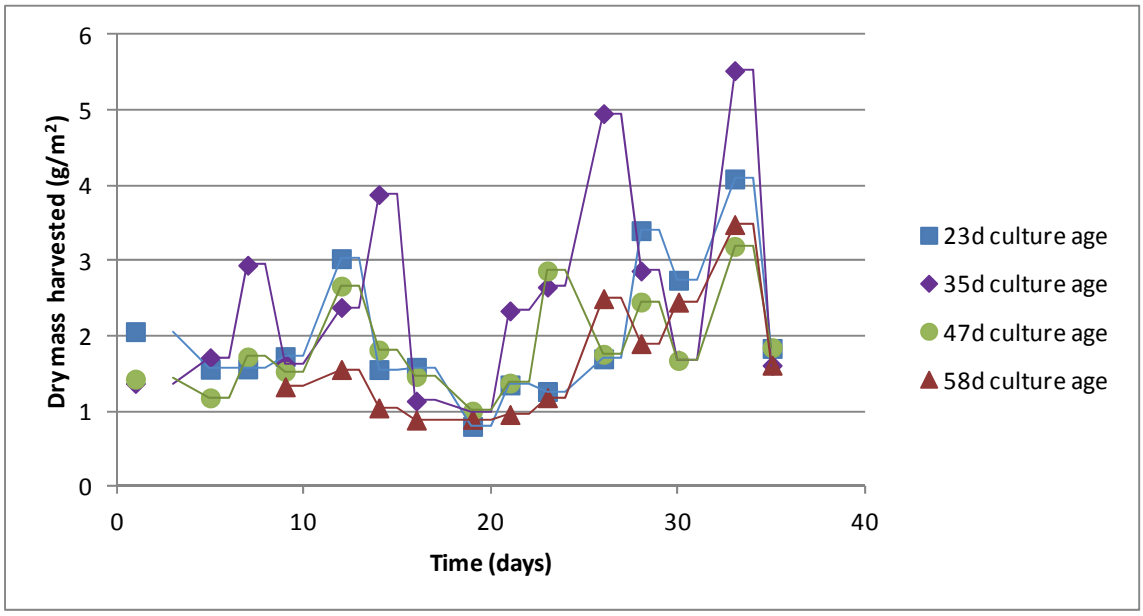

Figure 2-21: Dry mass of duckweed harvested to maintain culture ages at 18°C in a 1/100 Huttner solution

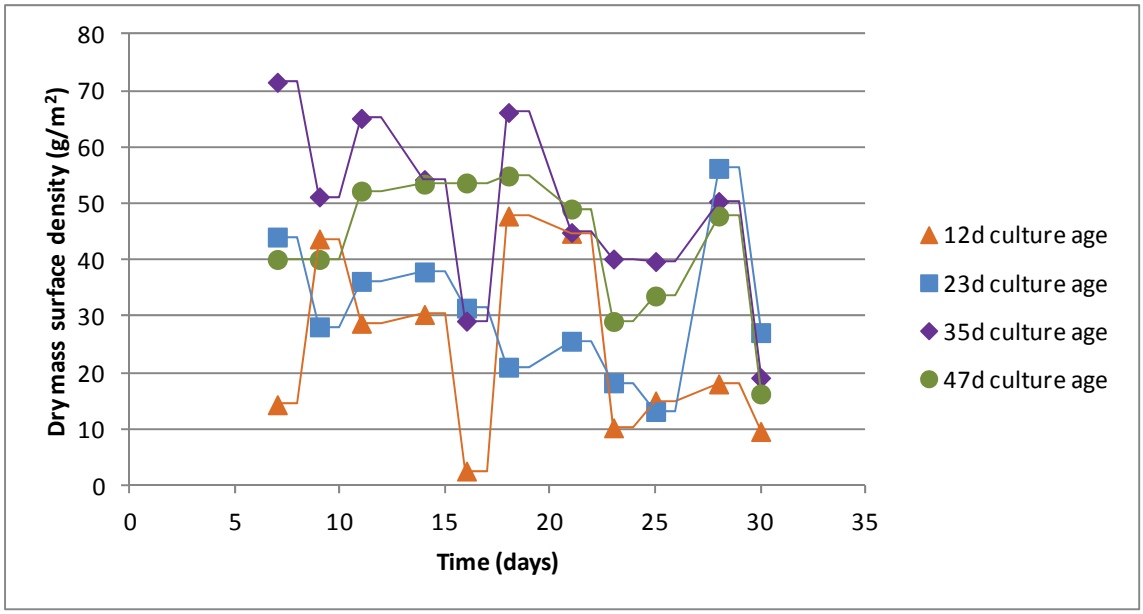

Figure 2-22: Dry mass surface density of different duckweed culture ages grown at 18°C in a 1/150 Huttner solution

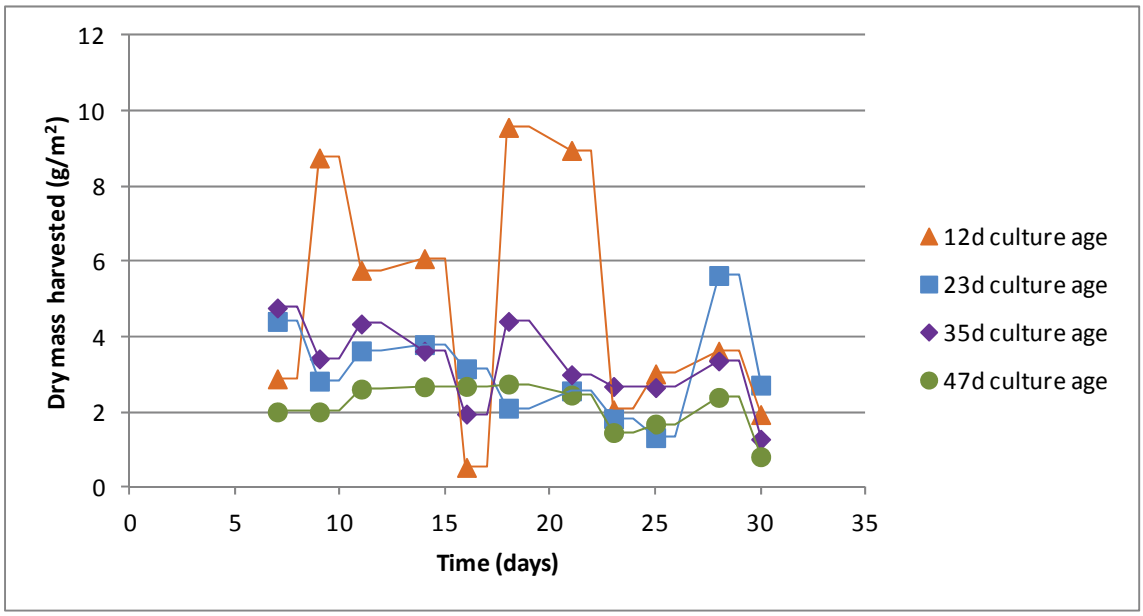

Figure 2-23: Dry mass of duckweed harvested to maintain culture ages at 18°C in a 1/150 Huttner solution

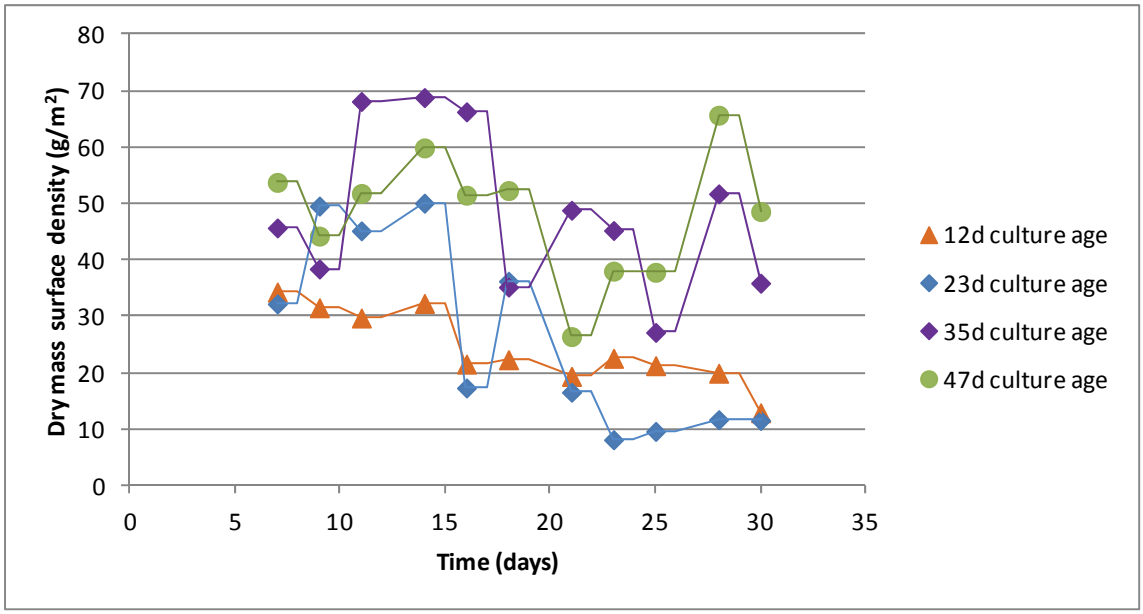

Figure 2-24: Dry mass surface density of different duckweed culture ages grown at 18°C in a 1/200 Huttner solution

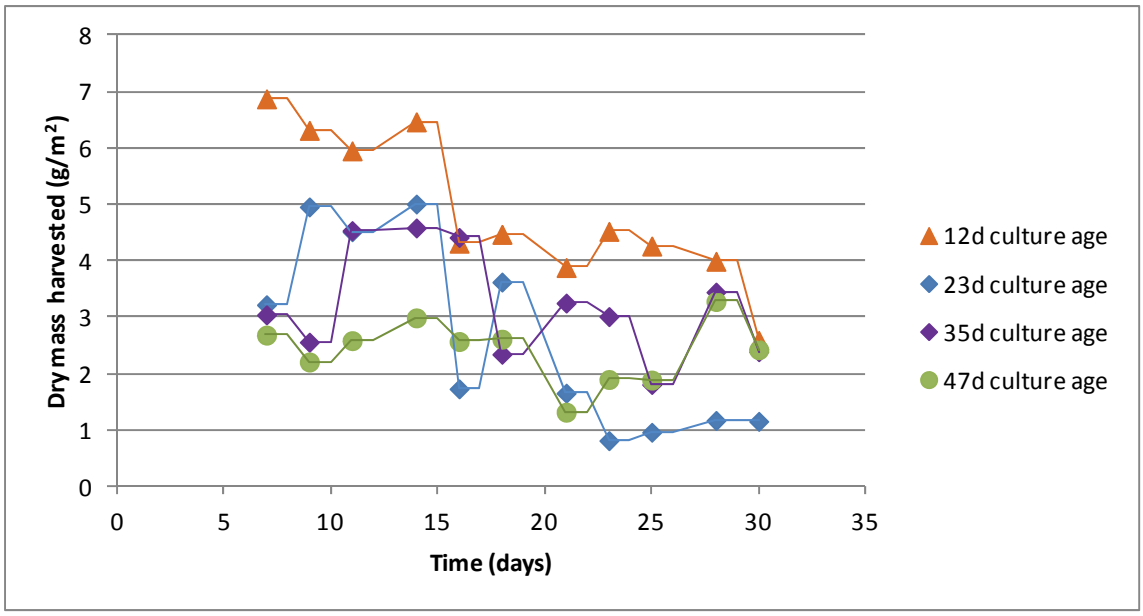

Figure 2-25: Dry mass of duckweed harvested to maintain culture ages at 18°C in a 1/200 Huttner solution

2.3.1.1.3 Growth rate at 13°C

The dry mass surface densities for different dilutions of Huttner media for the 13°C reactor are presented in Figure 2-26, Figure 2-28, Figure 2-30, Figure 2-32and Figure 2-34, and the dry masses of duckweed harvested in order to maintain the culture ages as indicated are presented in Figure 2-27, Figure 2-29, Figure 2-31, Figure 2-33 and Figure 2-35. Photographs of the surface areas of each chamber over time for each concentration at 25°C are presented

in Table A-11, Table A-12, Table A-13 and Table A-14 in Appendix A. A summary of the results is presented in Table 2-6.

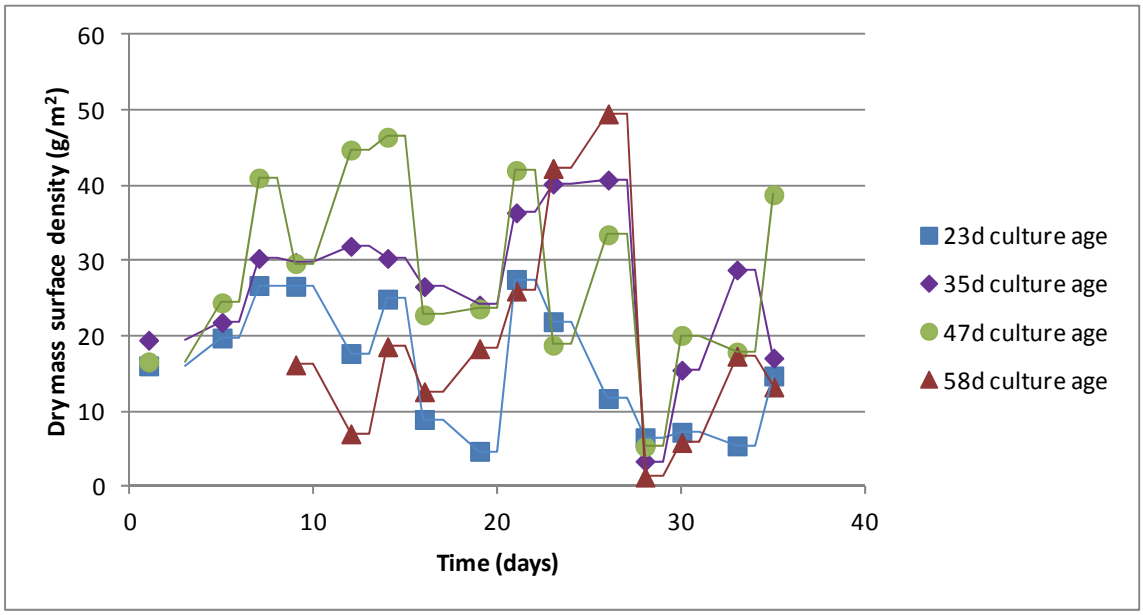

Figure 2-26: Dry mass surface density of different duckweed culture ages grown at 13°C in a 1/5 Huttner solution

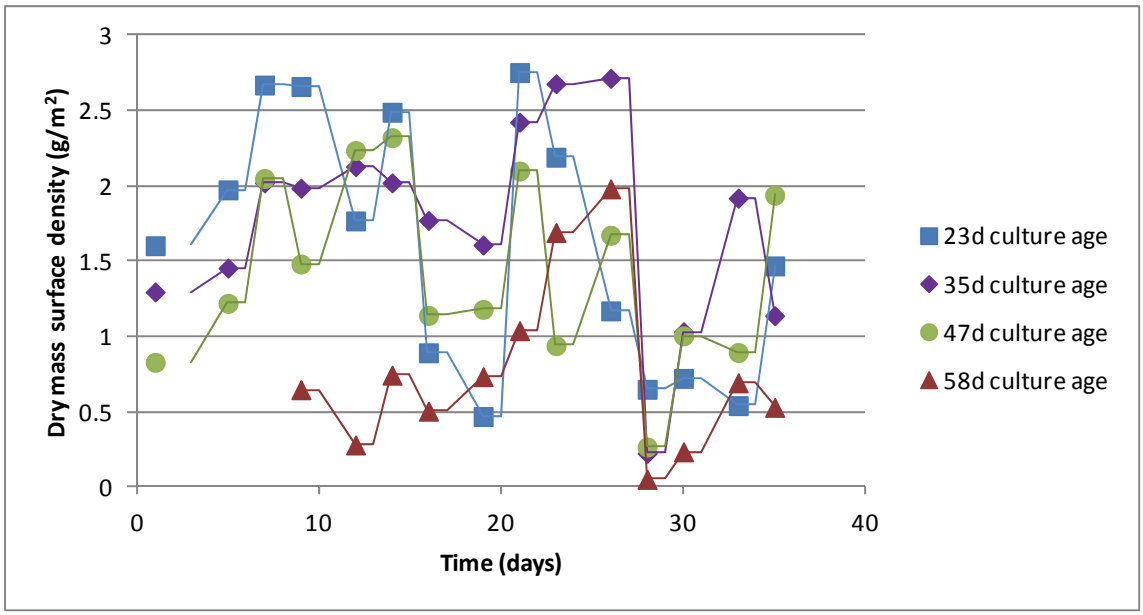

Figure 2-27: Dry mass of duckweed harvested to maintain culture ages at 13°C in a 1/5 Huttner solution

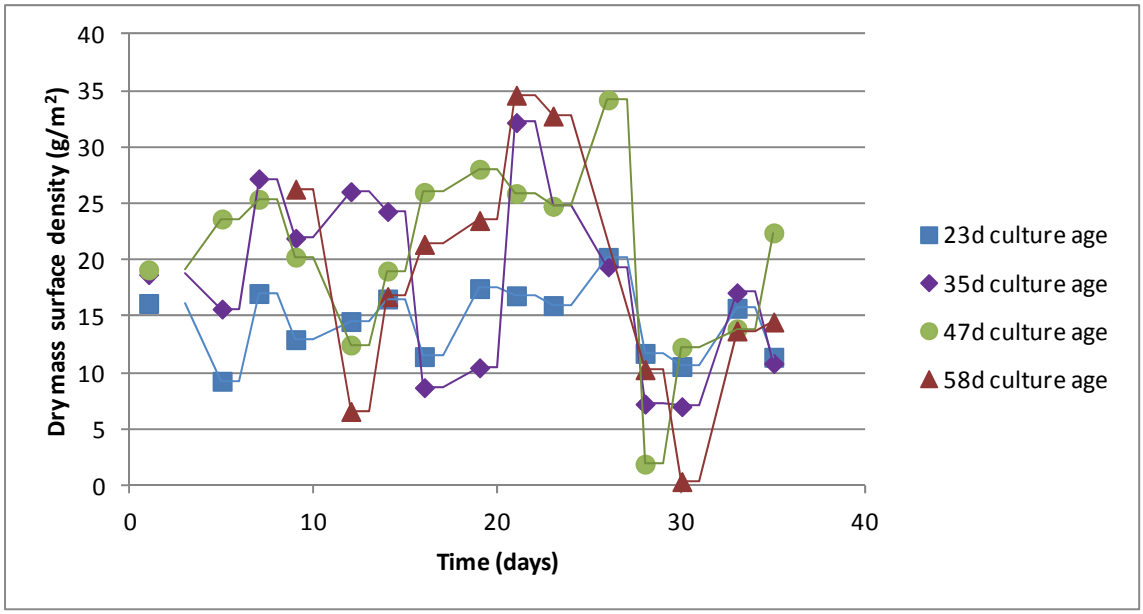

Figure 2-28: Dry mass surface density of different duckweed culture ages grown at 13°C in a 1/25 Huttner solution

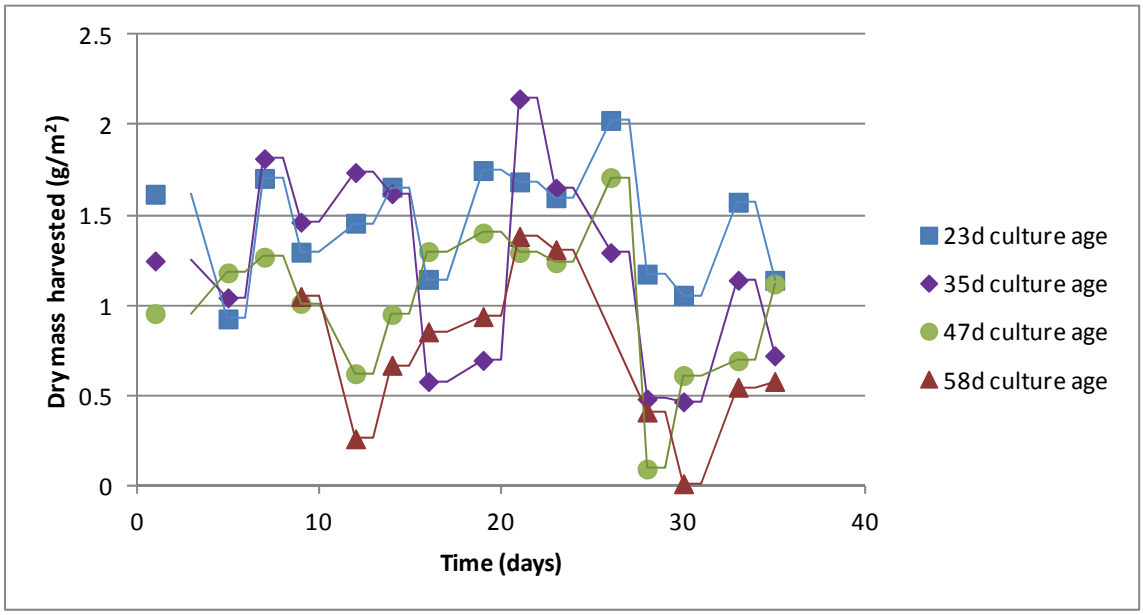

Figure 2-29: Dry mass of duckweed harvested to maintain culture ages at 13°C in a 1/25 Huttner solution

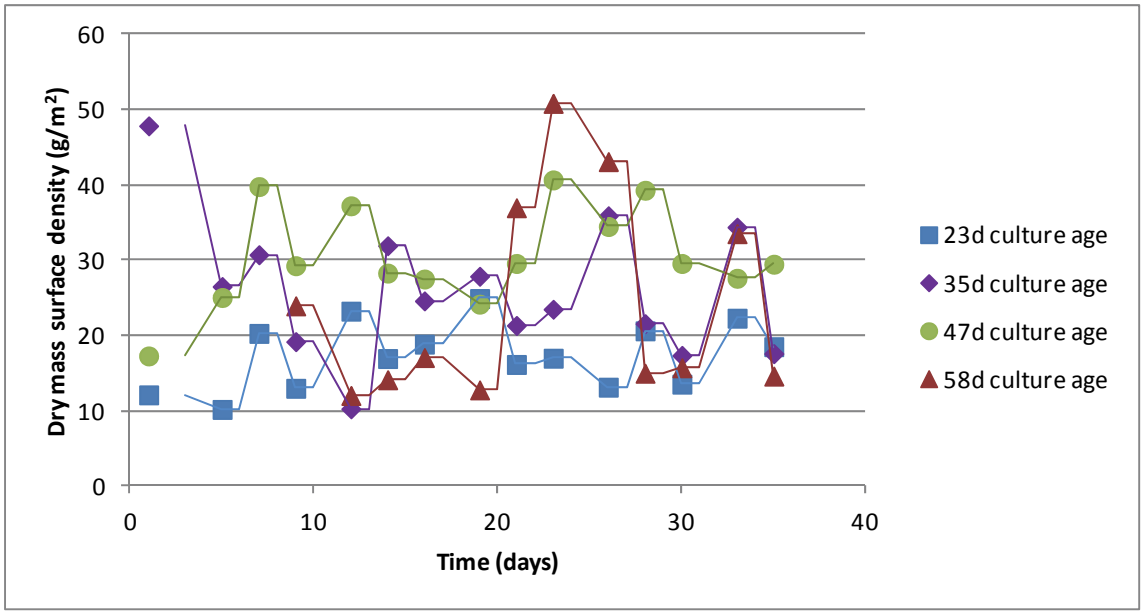

Figure 2-30: Dry mass surface density of different duckweed culture ages grown at 13°C in a 1/100 Huttner solution

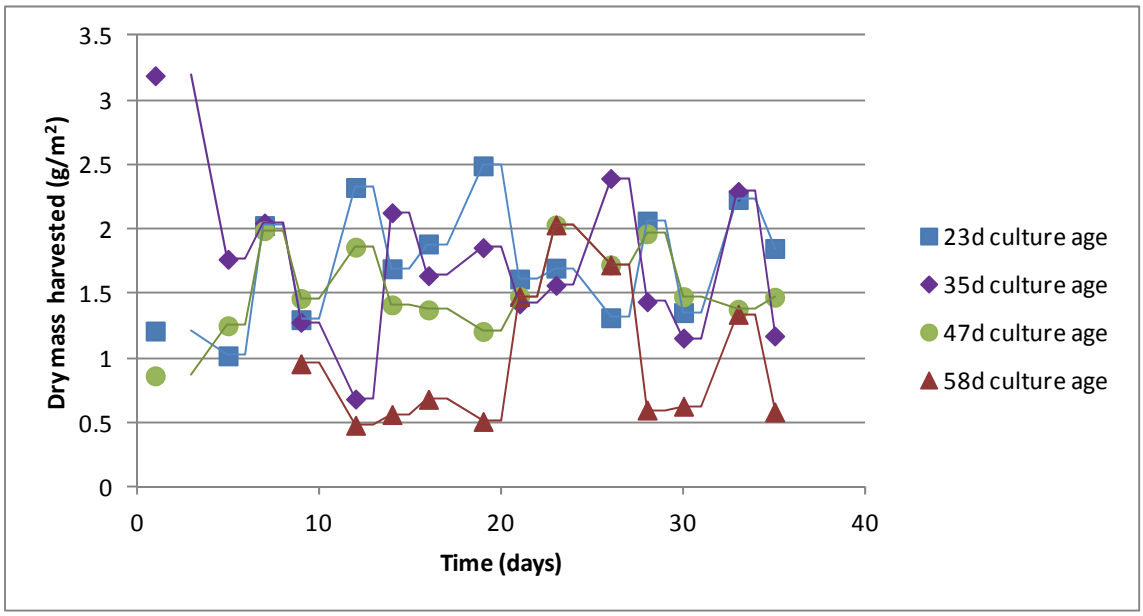

Figure 2-31: Dry mass of duckweed harvested to maintain culture ages at 13°C in a 1/100 Huttner solution

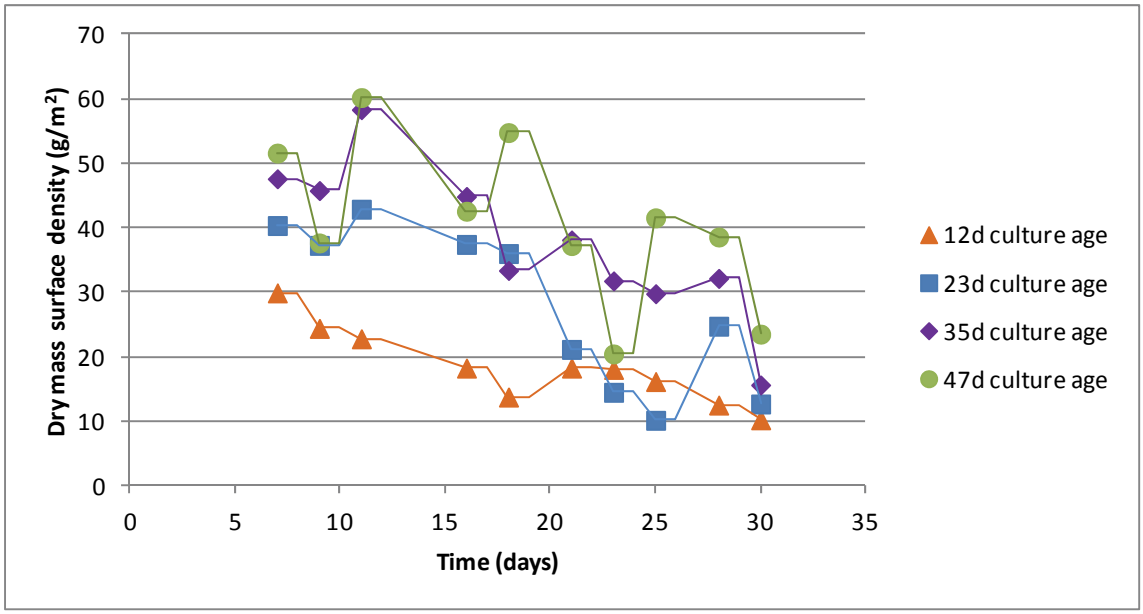

Figure 2-32: Dry mass surface density of different duckweed culture ages grown at 13°C in a 1/150 Huttner solution

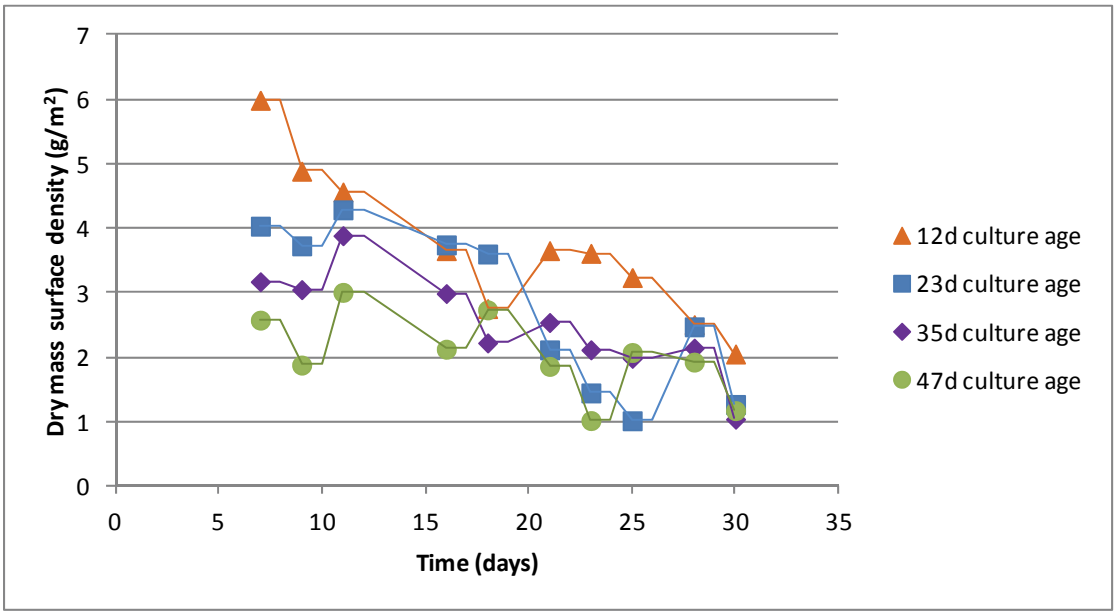

Figure 2-33: Dry mass of duckweed harvested to maintain culture ages at 13°C in a 1/150 Huttner solution

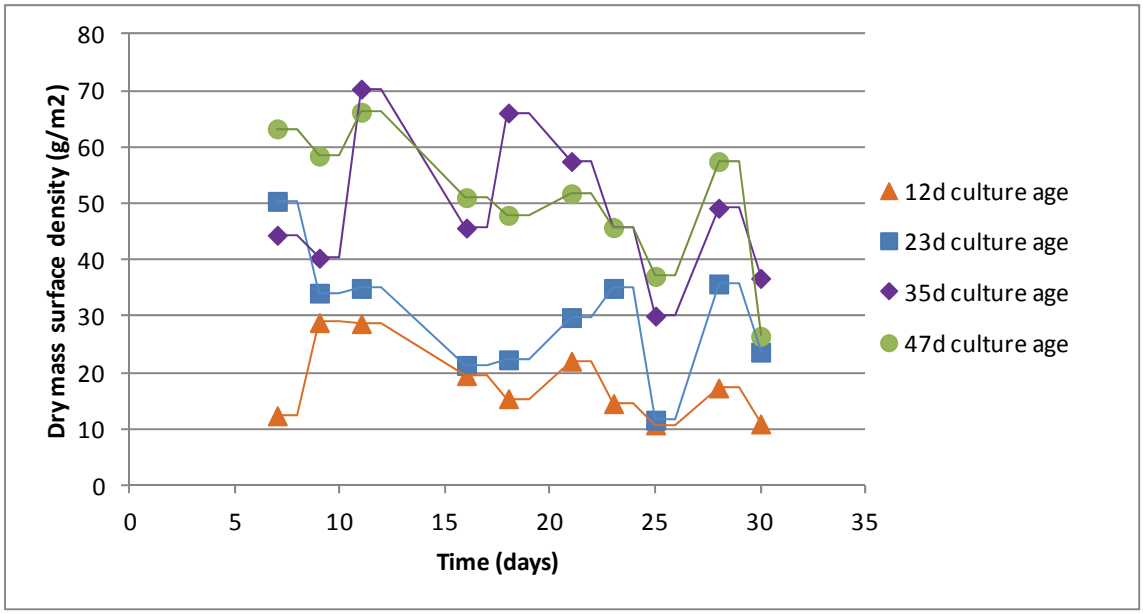

Figure 2-34: Dry mass surface density of different duckweed culture ages grown at 13°C in a 1/200 Huttner solution

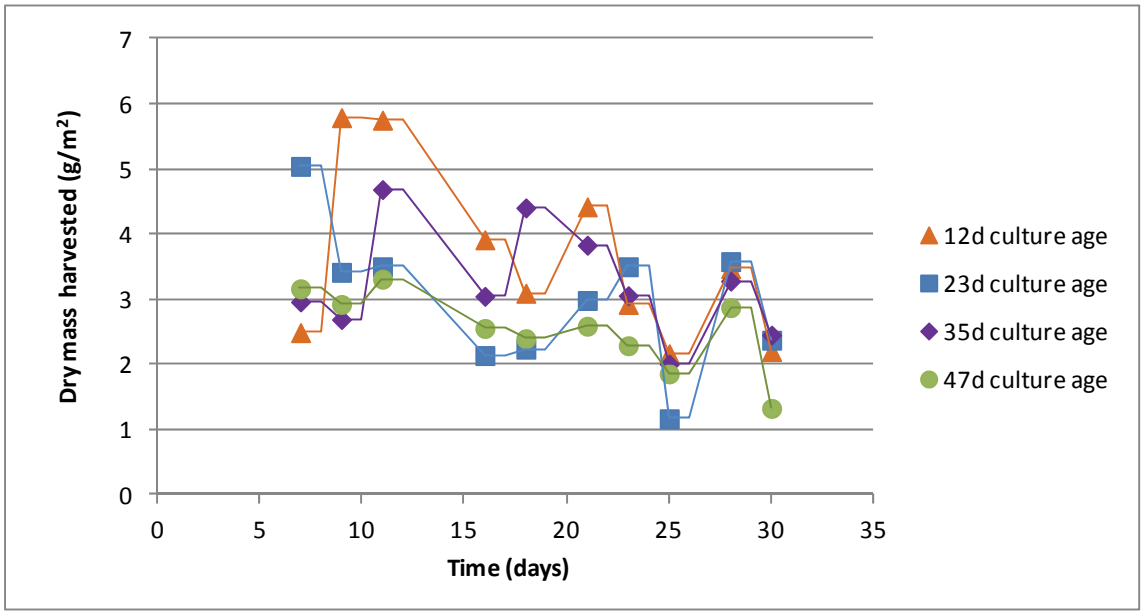

Figure 2-35: Dry mass of duckweed harvested to maintain culture ages at 13°C in a 1/200 Huttner solution

As indicated above, a summary of the effect of harvesting, nutrient concentration and temperature on the surface area coverage of duckweed in the reactor chambers expressed as dry mass is presented in Table 2-6. At a 1/5 Huttner dilution, all three reactors showed a complete wash out of duckweed at all culture ages. The 1/25 Huttner dilution showed a decrease in the surface density in the reactors at 13°C and 18°C at all culture ages, but a steady surface density in the 12d and 23d culture ages at 25°C, and an increase at the 35d and 47d culture ages. At a 1/100 Huttner dilution all culture ages showed an increase in

surface area density at 25°C, as well as at 18°C in the 23d, 35d, 47d and 58d culture ages. Steady surface densities were noted for the 35d, 47d and 58d culture ages at 13°C, whereas a decrease in the surface density was observed at a 12d culture age at 18°C and 12 and 23d culture ages at 13°C. At a 1/150 Huttner dilution, all culture ages at 25°C showed an increase in dry mass surface density. With the exception of the 47d culture age at 18°C, all other culture ages at both 18°C and 13°C showed a decrease in surface density at this dilution. For a 1/200 Huttner dilution at 25°C, the 35d culture age showed a steady surface density, while 12d, 23d and 47d all showed an increase. All culture ages in both the 13°C and 18°C reactors showed a decrease in surface density with the exception of a steady surface density observed at the 23d age at 13°C.

Table 2-6: Indication of a net increase, decrease, or stable duckweed surface density, at different temperatures, solution concentrations and harvesting rates

+; Increase, ≈; stable density, –; decrease

Temperature	Culture Age (d)	Huttner Media Dilution				
		1/5	1/25	1/100	1/150	1/200
25°C	12	–	≈	+	+	+
	23	–	≈	+	+	+
	35	–	+	+	+	≈
	47	–	+	+	+	+
18°C	12			–	–	–
	23	–	–	+	–	–
	35	–	–	+	–	–
	47	–	–	+	≈	–
	58	–	–	+		
13°C	12			–	–	–
	23	–	–	–	–	≈
	35	–	–	≈	–	–
	47	–	–	≈	–	–
	58	–	–	≈		

2.3.1.1.4 Growth rate in shade

The average light intensities measured at 10:00am, 12:00pm and 3:00pm were 6616.3lux, 8325.3lux and 6687.9lux respectively for the period of the experiment. The mid-day light intensity was approximately double that of the light intensity in the temperature controlled rooms, and was approximately half the saturation light intensity of 18500lux reported for

Lemna spp. (Lasfar et al., 2007). The average temperature in the reactor in the shade was 24.8°C.

The dry mass surface densities for different dilutions of Huttner media for the reactor set up in the shade are presented in Figure 2-36, Figure 2-38 and Figure 2-40, and the dry masses of duckweed harvested in order to maintain the culture ages as indicated are presented in Figure 2-37, Figure 2-39 and Figure 2-41. Photographs of the surface areas of each chamber over time for each concentration in the shade are presented in Table A-16, Table A-17 and Table A-18 in Appendix A.

Moving average trendlines have been included in the figures to illustrate the changes in surface density with time. Using linear trendlines, it was determined whether there was a net increase, decrease or a stable dry mass surface area in the chambers at different nutrient concentrations and harvesting rates once steady state was reached. A summary of the results is presented in Table 2-7.

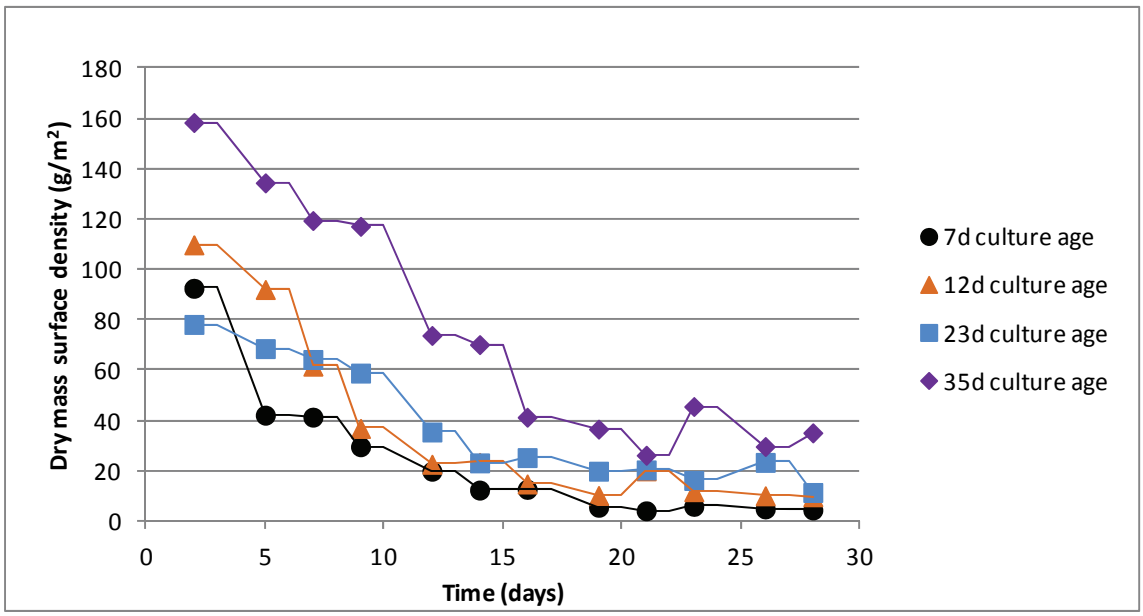

Figure 2-36: Dry mass surface density of different duckweed culture ages in the shade in a 1/5 Huttner solution

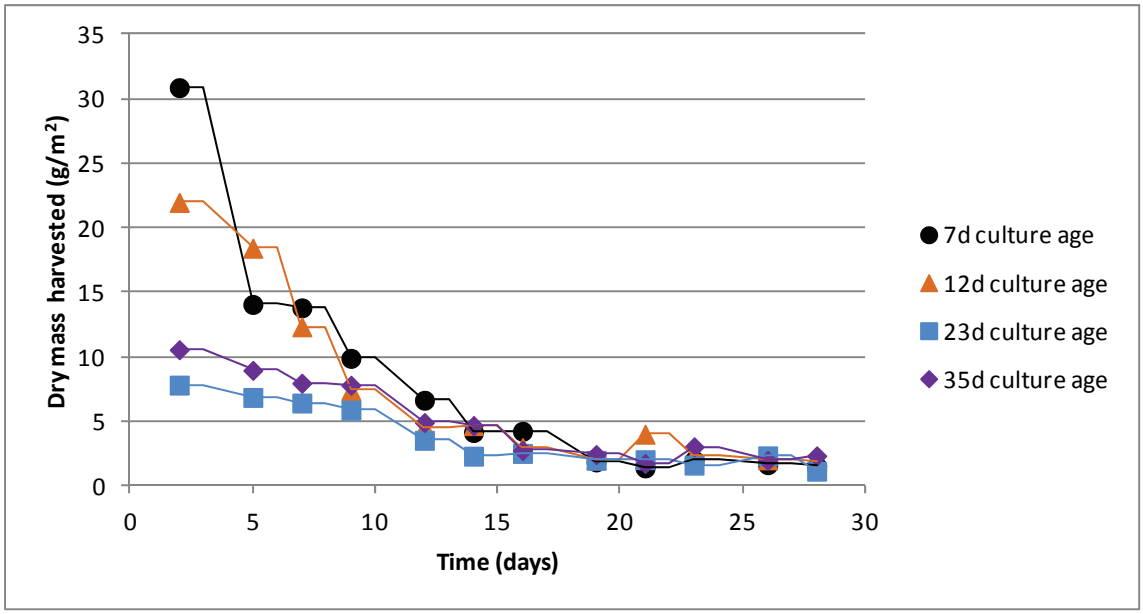

Figure 2-37: Dry mass of duckweed harvested to maintain culture ages in the shade in a 1/5 Huttner solution

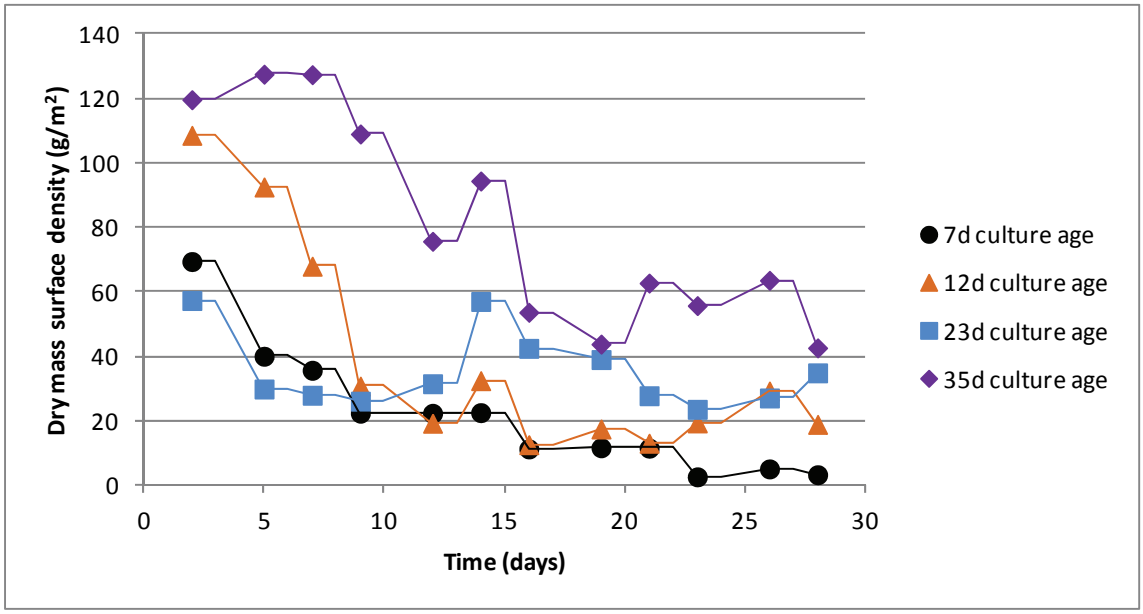

Figure 2-38: Dry mass surface density of different duckweed culture ages in the shade in a 1/25 Huttner solution

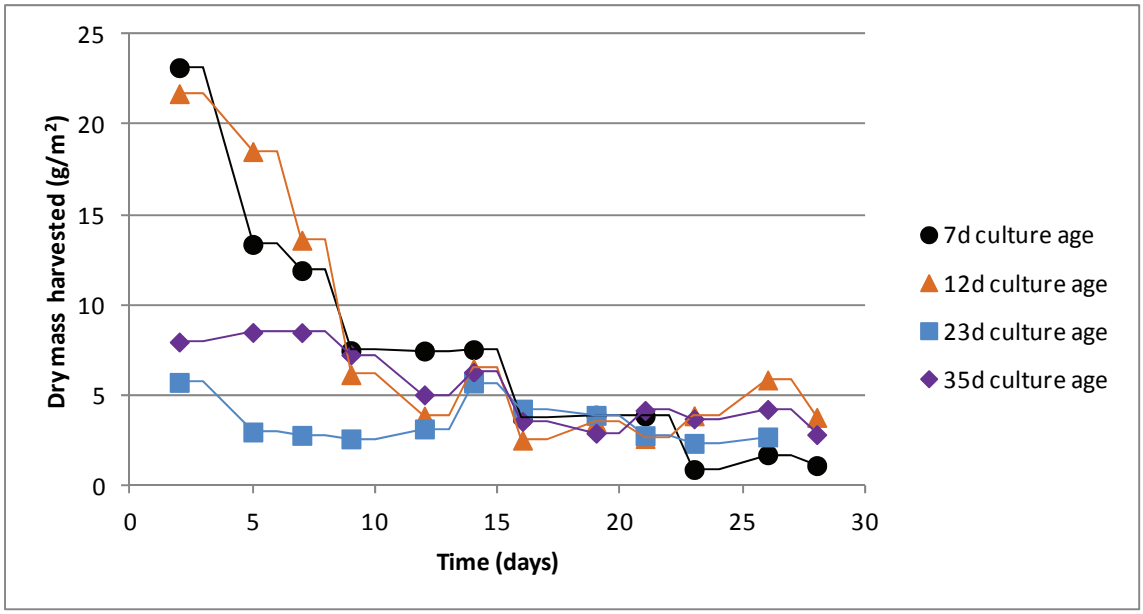

Figure 2-39: Dry mass of duckweed harvested to maintain culture ages in the shade in a 1/25 Huttner solution

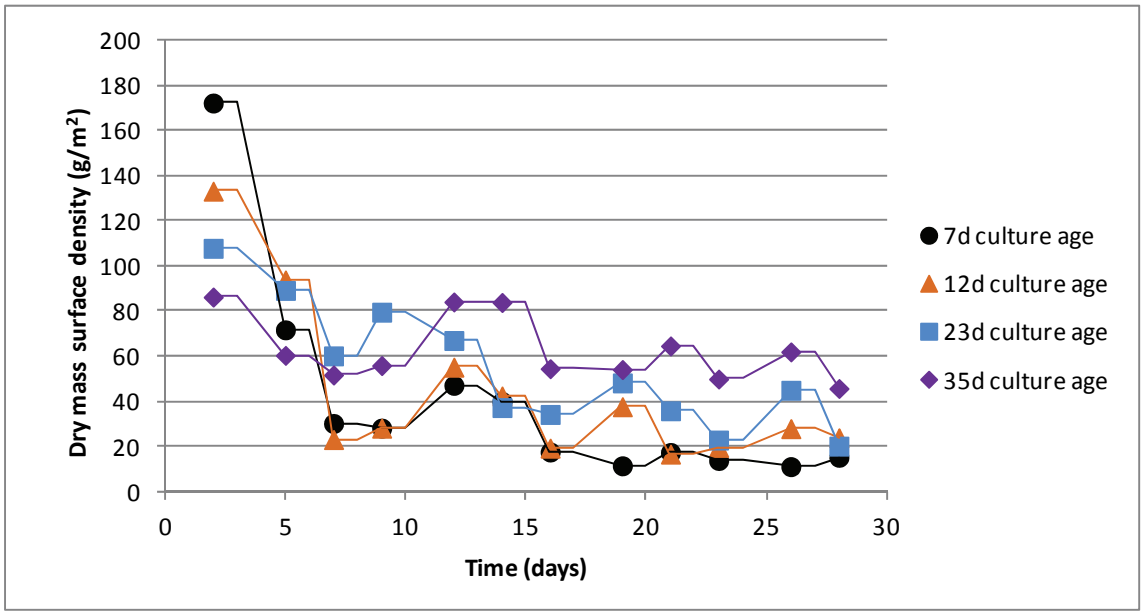

Figure 2-40: Dry mass surface density of different duckweed culture ages grown in the shade in a 1/100 Huttner solution

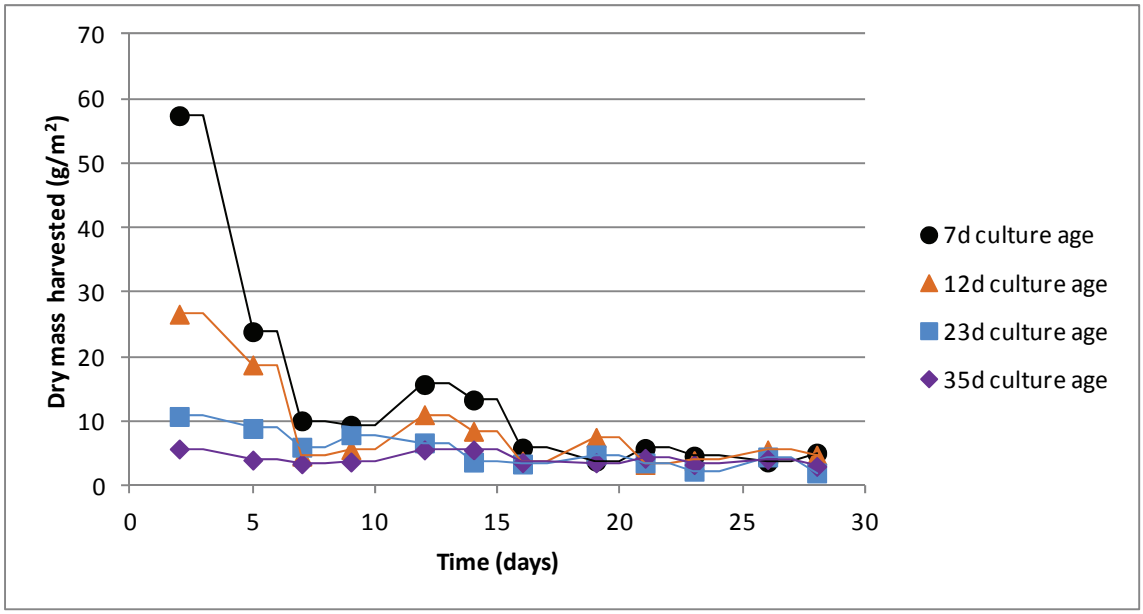

Figure 2-41: Dry mass of duckweed harvested to maintain culture ages in the shade in a 1/100 Huttner solution

2.3.1.1.5 Growth rate in sun

The average light intensities measured at 10:00am, 12:00pm and 3:00pm were 67452.6lux 86234.7lux and 53278.9lux respectively for the period of the experiment, which was in the order of 10 times higher than that observed in the shade. The average light intensities observed far exceeded the saturation light intensity of 18500lux reported for *Lemna* spp (Lasfar et al., 2007); and in fact the average light intensities at 10:00am and 12:00pm exceeded 1200 $\mu E.m^{-2}.s^{-1}$ (64800lux) which was the light intensity at which *Lemna minor* cultures have been found to be photo inhibited (Wedge & Burris, 1982). The average temperature in the reactor in the sun was 27.8°C.

The dry mass surface densities for different dilutions of Huttner media for the reactor set up in the sun are presented in Figure 2-42, Figure 2-45 and Figure 2-47, and the dry masses of duckweed harvested in order to maintain the culture ages as indicated are presented in Figure 2-43, Figure 2-46 and Figure 2-47. Photographs of the surface areas of each chamber over time for each concentration in the sun are presented in Table A-19, Table A-20 and Table A-21 in Appendix A. A summary of the results is presented in Table 2-7.

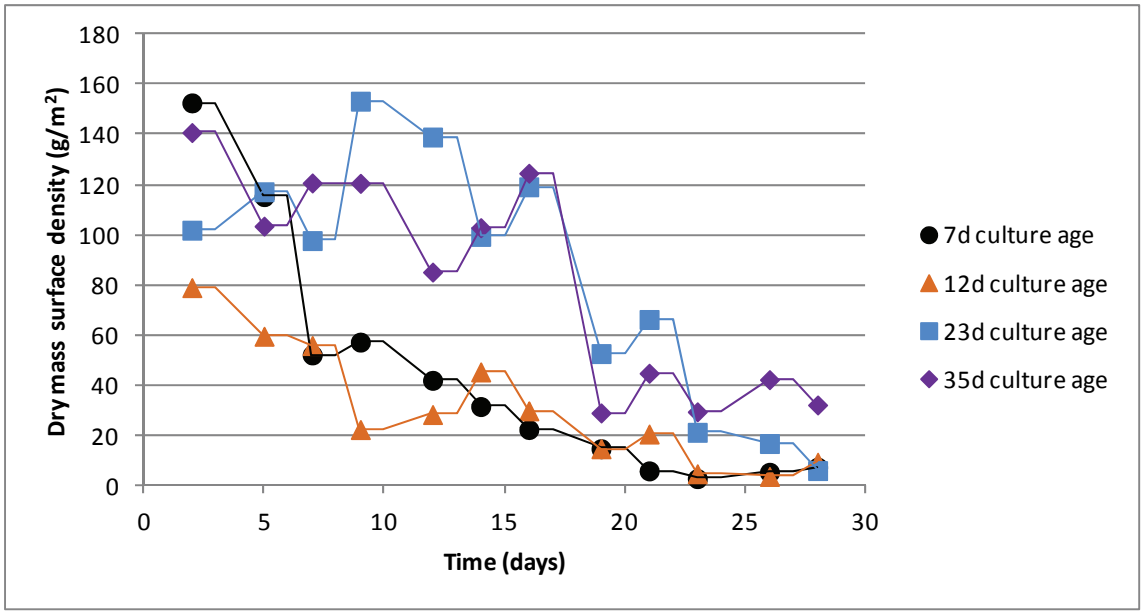

Figure 2-42: Dry mass surface density of different duckweed culture ages in the sun in a 1/5 Huttner solution

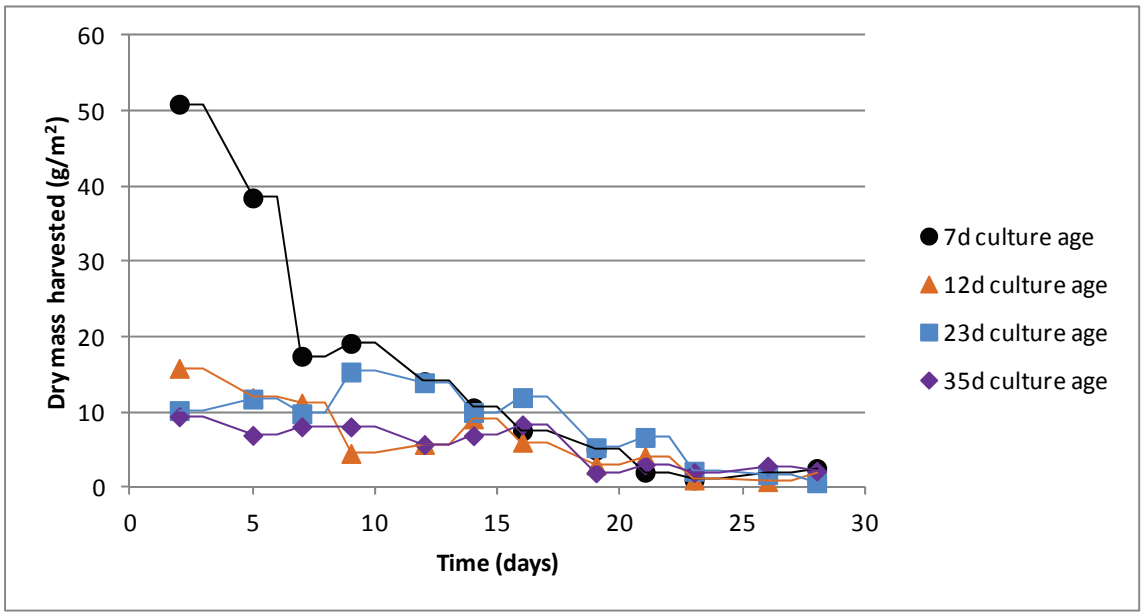

Figure 2-43: Dry mass of duckweed harvested to maintain culture ages in the sun in a 1/5 Huttner solution

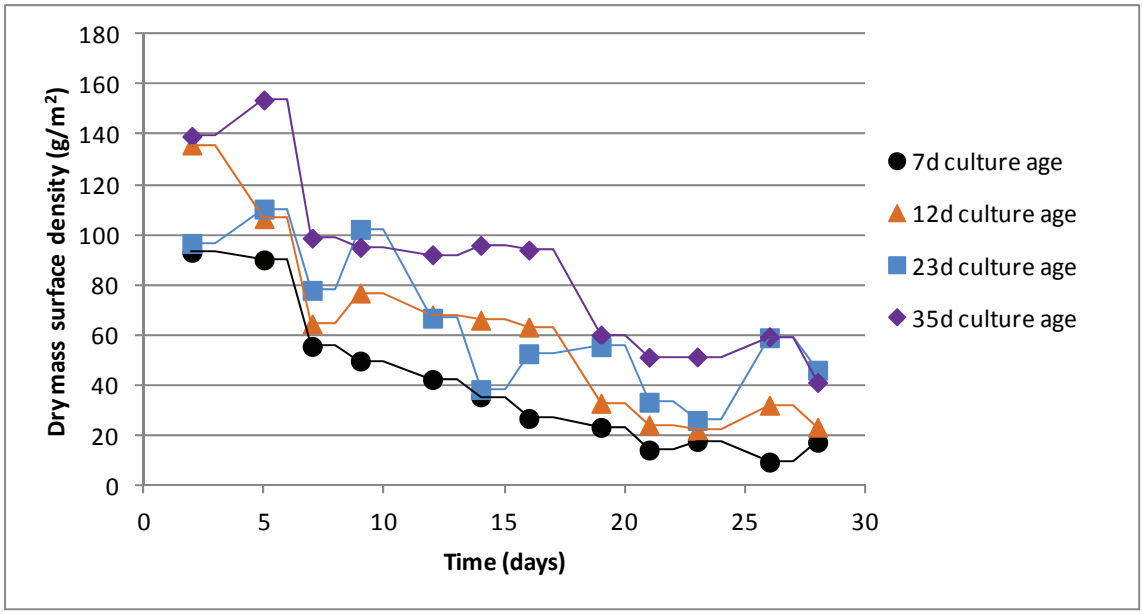

Figure 2-44: Dry mass surface density of different duckweed culture ages in the sun in a 1/25 Huttner solution

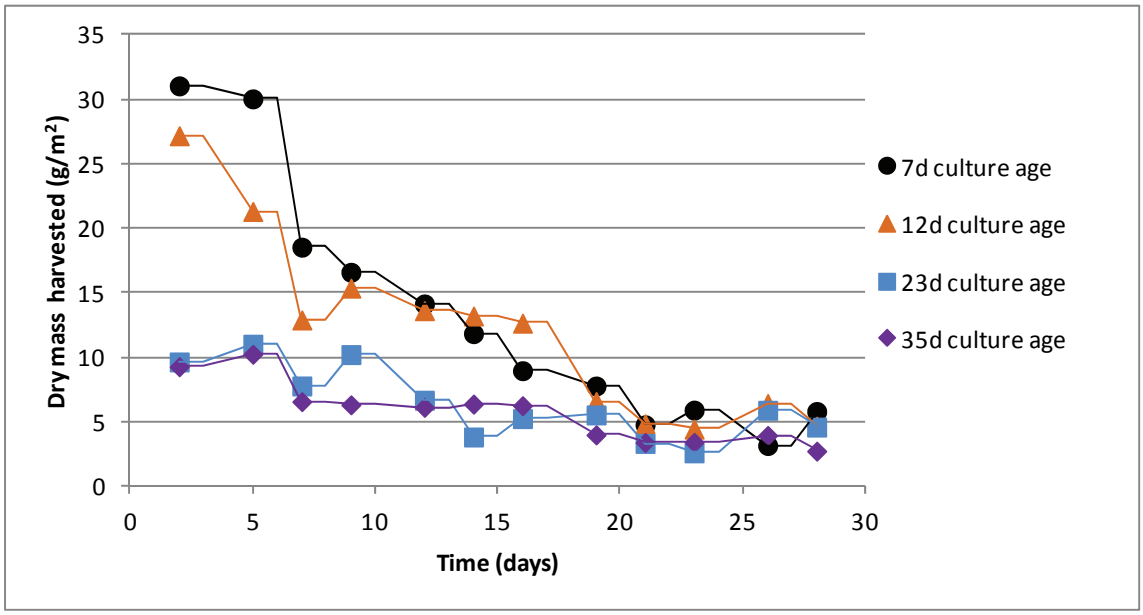

Figure 2-45: Dry mass of duckweed harvested to maintain culture ages in the sun in a 1/25 Huttner solution

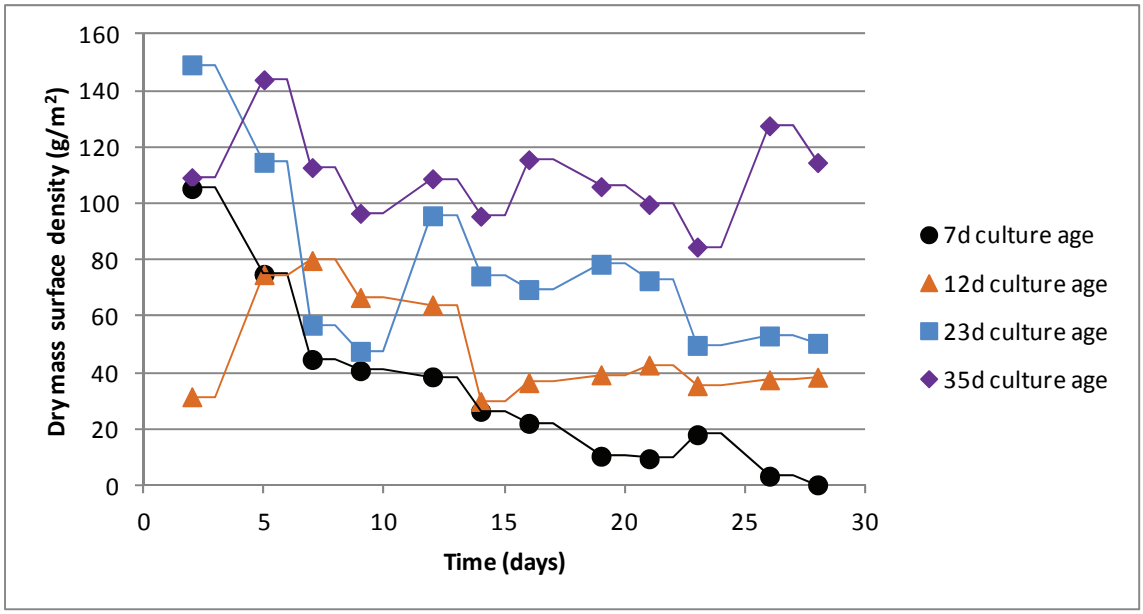

Figure 2-46: Dry mass surface density of different duckweed culture ages grown in the sun in a 1/100 Huttner solution

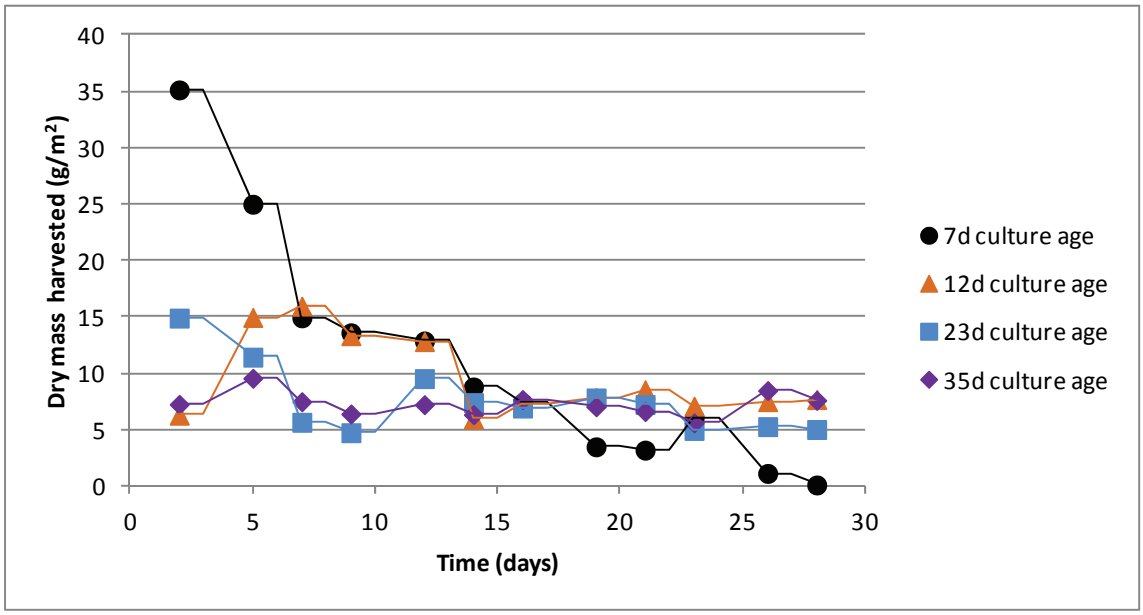

Figure 2-47: Dry mass of duckweed harvested to maintain culture ages in the sun in a 1/100 Huttner solution

As indicated above, a summary of the effect of harvesting, nutrient concentration and temperature on the surface area coverage of duckweed in the reactor chambers expressed as dry mass is presented in Table 2-7. At a 1/5 Huttner dilution, both the reactors (sun and shade) showed a wash out of duckweed at the 7d, 12d and 23d culture ages, and steady surface density in the 35d culture age. At a 1/25 Huttner dilution, both reactors showed a decrease in the surface density in the reactors for the 7d culture age, but a steady surface density in the 12d and 23d and 35d culture ages. The same pattern was seen in the 1/100

dilution for both reactors, with the exception of the 35d culture age in the sun that showed an increase in surface density.

Table 2-7: Indication of a net increase, decrease, or stable duckweed surface density, in the shade and sun at different culture ages and harvesting rates

+; Increase, ≈; stable density, –; decrease

Condition	Culture Age (d)	Huttner Media Dilution		
		1/5	1/25	1/100
Shade	7	–	–	–
	12	–	≈	≈
	23	–	≈	≈
	35	≈	≈	≈
Sun	7	–	–	–
	12	–	≈	≈
	23	–	≈	≈
	35	≈	≈	+

2.3.1.1.6 Biomass composition

The composition of the dry duckweed biomass harvested under conditions of controlled temperature, light intensity and different nutrient concentrations is presented in Table 2-8, and the composition of the dry duckweed biomass harvested under conditions of uncontrolled light intensity and temperature in the shade and sun is presented in Table 2-9.

A two-factor ANOVA analysis without replication was conducted on the data, with $\alpha = 0.05$ and it was found with greater than 95% confidence that the difference in the biomass nutrient composition between Huttner media dilutions was not significant for any of the temperatures or light intensities tested ($F_{2,8} = 0.58$, $P = 0.58$ for COD; $F_{2,8} = 0.23$, $P = 0.58$ for TP; $F_{2,8} = 0.94$, $P = 0.42$ for TKN). However, there was a significant difference in both the TKN and total P concentrations ($F_{4,8} = 7.27$, $P = 0.008$ for TP; $F_{4,8} = 3.92$, $P = 0.047$ for TKN) with the highest nitrogen and phosphorus concentrations observed in the plants grown in the sunlight and the lowest in the plants grown at 13°C. Temperature did not affect the COD concentration ($F_{4,8} = 2.43$, $P = 0.13$).

The nutrient compositions of the plants grown at 25°C under controlled light intensity was compared with those grown in the shade, where the temperature was also 25°C but the light intensity was double of that of the temperature controlled rooms and it was found that the concentrations of total P and TKN were not significantly different ($F_{1,2} = 4.23$, $P = 0.18$ for TP;

$F_{1,2}$ = 0.96, P = 0.42 for TKN), but that there was a significant difference in the COD concentration ($F_{1,2}$ = 78.57, P = 0.012) between plants, with the highest COD concentration in the plants grown in the shade. A similar trend was noted when comparing the nutrient compositions of plants grown in the sun when compared with plants in the shade; concentrations of total P and TKN were not significantly different ($F_{1,2}$ = 0.86, P = 0.45 for TP; $F_{1,2}$ = 2.63, P = 0.24 for TKN), but that there was a significant difference in the COD concentration ($F_{1,2}$ = 39.5, P = 0.024)

Table 2-8: Composition of dry biomass from different conditions of controlled temperate and nutrient media concentration and light intensity

Temperature	Biomass parameter (g/kg dry mass)	Huttner Media Dilution				
		1/5	1/25	1/100	1/150	1/200
25°C	COD	1250	1172	1179	1369	1099
	Total P	10.3	8.8	6.2	8.0	8.3
	TKN	56	55	49	42.3	45
18°C	COD	1093	1162	1520	1423	1299
	Total P	4.5	7.0	5.1	7.7	6.6
	TKN	38	40	33	46	52.5
13°C	COD	1398.9	2150	1316	1200	1163
	Total P	4.8	5.2	6.6	7.1	6.2
	TKN	30	42	40	60	65

Table 2-9: Composition of dry biomass from different conditions of temperate and nutrient media concentration in natural light conditions

Condition	Biomass parameter (g/kg dry mass)	Huttner Media Dilution		
		1/5	1/25	1/100
Shade	COD	1541.8	1571.2	1465.3
	Total P	15.8	9.2	9.4
	TKN	57	54	58
Sun	COD	1767.1	1733.3	1597.1
	Total P	14.9	13.1	10.3
	TKN	56.3	41.7	25

2.3.1.2 Effect of nutrient concentration and light intensity on population species composition and plant physiology

2.3.1.2.1 Species composition

Within a few days, all the *Lemna gibba* plants disappeared from the stock culture. The *Wolffia* plants were rapidly washed out at 13°C and 18°C in all concentrations of Huttner media, and only the *Lemna turionifera* plants remained at these temperatures. At 25°C, the *Wolffia* plants were more numerous in the highest nutrient concentration (1/5 Huttner media) than the *L. turionifera*, although were almost absent at the lower nutrient concentrations tested.

A mixed culture was used to seed the reactors in the shade and the sun. Within 1 week *Wolffia* plants became dominant in all Huttner media dilutions in the shade, whereas *Lemna turionifera* became dominant in all concentrations tested in the sun. The *Wolffia* plants did not completely wash out of the chambers containing the 1/5 dilution, but disappeared almost completely from the 1/25 and 1/100 dilutions. Pictures of the species composition in the 1/25 nutrient dilution in the shade and the sun after 12d is illustrated in Figure 2-48.

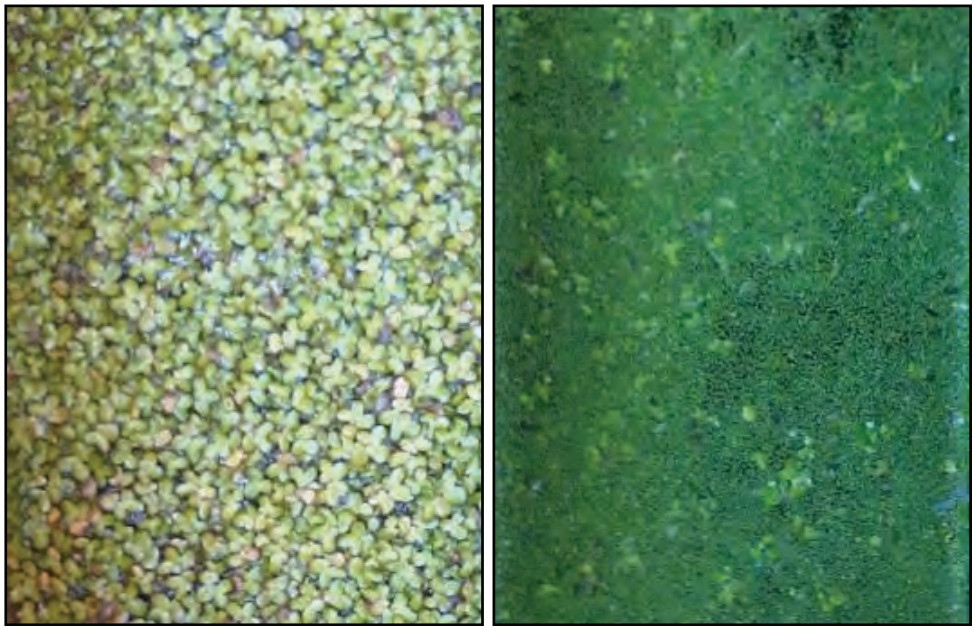

Figure 2-48: Differences in species composition between the sun (left) and shade (right) at 1/25 Huttner dilution after 19d

2.3.1.2.2 Plant physiology

The different nutrient concentrations affected the frond size and root length, with the largest fronds and longest roots being observed at the lower nutrient concentrations. The frond sizes and root lengths of duckweed plants grown at different dilutions of Huttner

media are shown in Figure 2-49. The roots of the *Lemna turionifera* plants grown at the 1/5 dilution were less than 1 cm, whereas those of plants grown in the 1/150 dilution were greater than 4 cm.

Although the root lengths increased in the duckweed plants grown at the lower dilutions at 18°C and 20°C, the frond sizes were greatly reduced in plants grown at all solution concentrations at these temperatures when compared with the plants grown at 25°C. This is illustrated in Figure 2-50, which shows the difference in frond size between plants grown for 30 days in 1/150 dilution at 25°C and 18°C. At 25°C, the moisture content of the plants decreased in the 1/100, 150 and 1/200 dilutions, with the greatest decrease in the 1/150 dilution (Figure 2-51). However, the moisture content remained constant in plants grown in these dilutions at 18° (Figure 2-52), and increased in plants grown at 13°C (Figure 2-53).

L. turionifera is a turion forming species. Turion formation was noted in the 18°C reactor at the 1/25 Huttner media dilution.

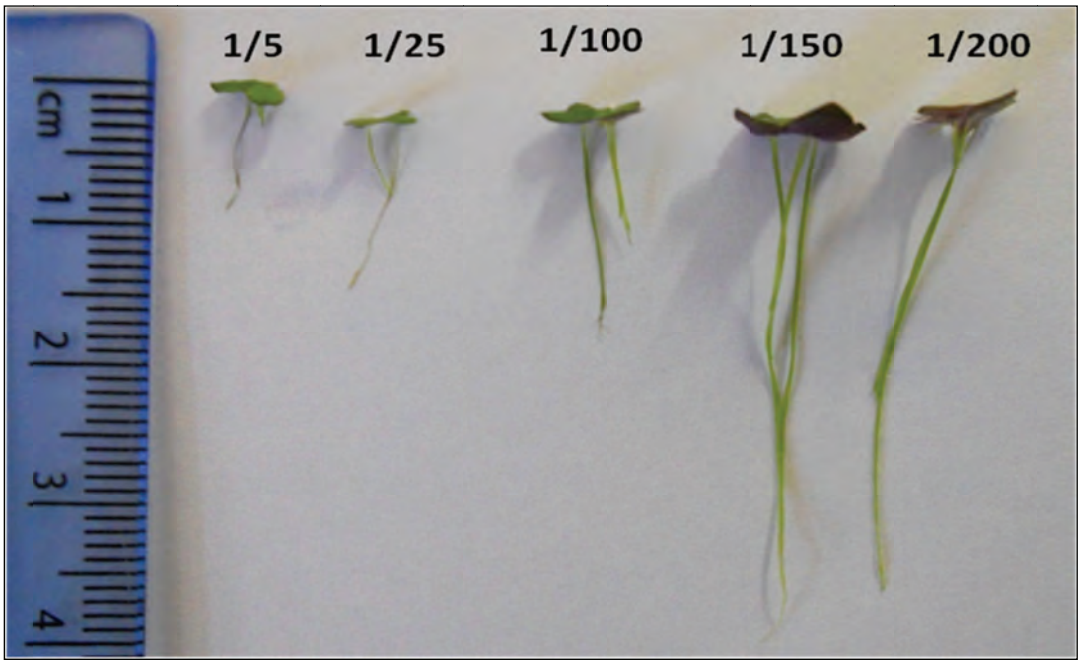

Figure 2-49: Root length and frond size at different Huttner media concentrations at 25°C

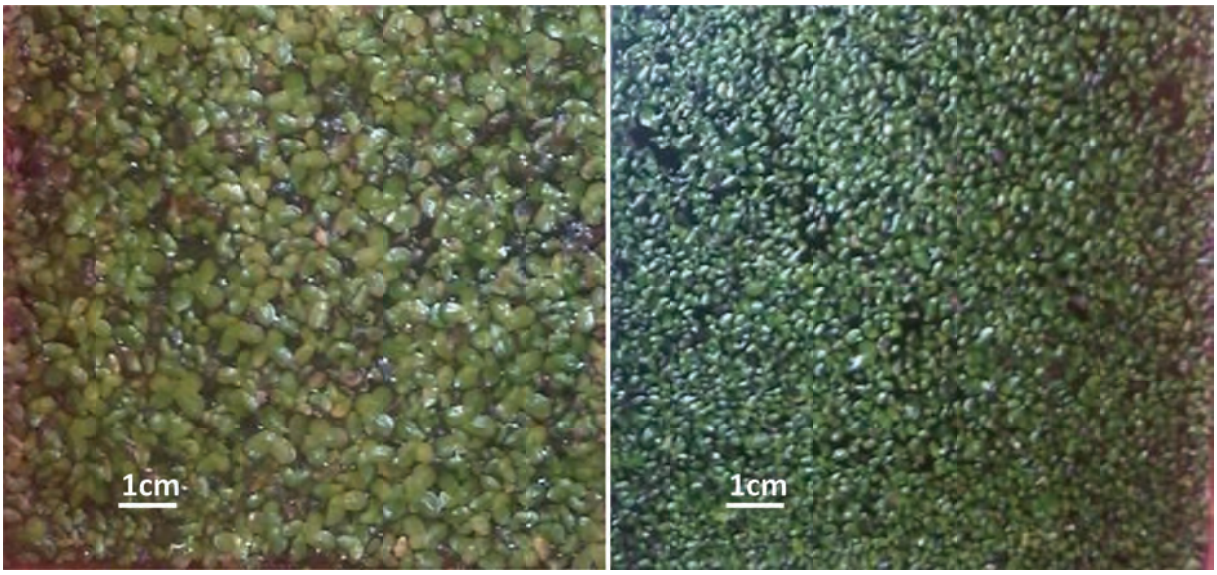

Figure 2-50: Frond sizes in 1/150 Huttner medium at 25°C (left) and at 18°C (right) after 30 days

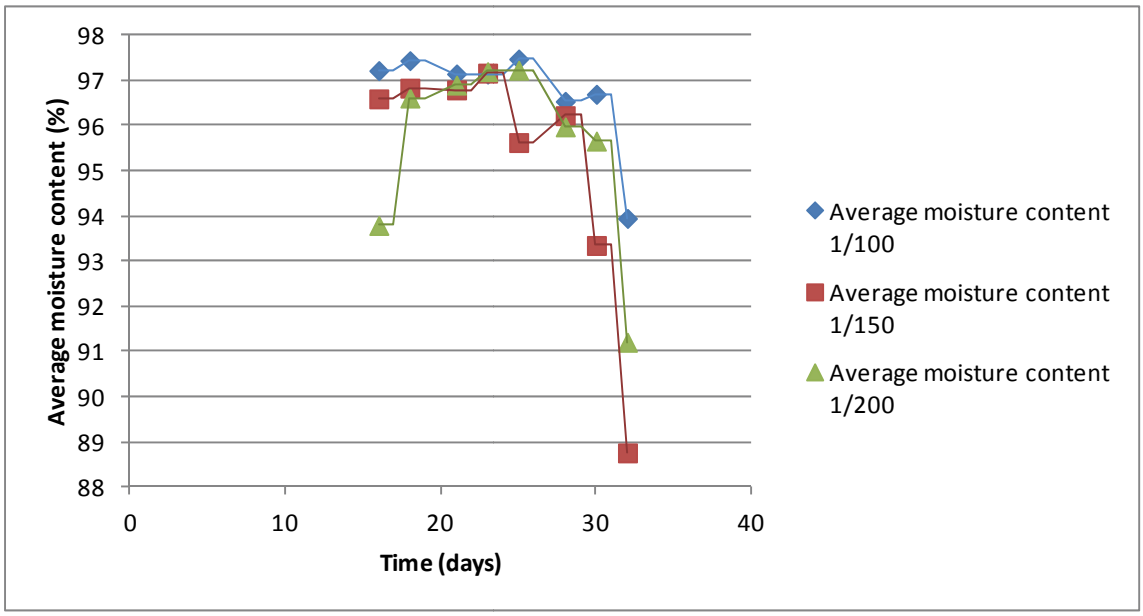

Figure 2-51: Average moisture content of duckweed grown in different Huttner media dilutions at 25°C

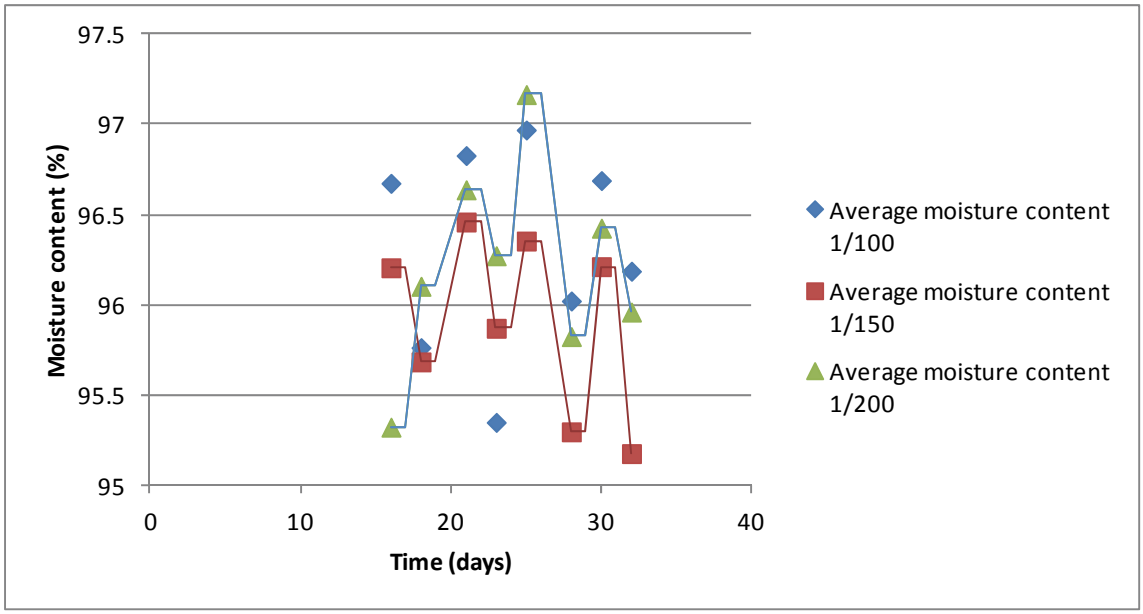

Figure 2-52: Average moisture content of duckweed grown in different Huttner media dilutions at 18°C

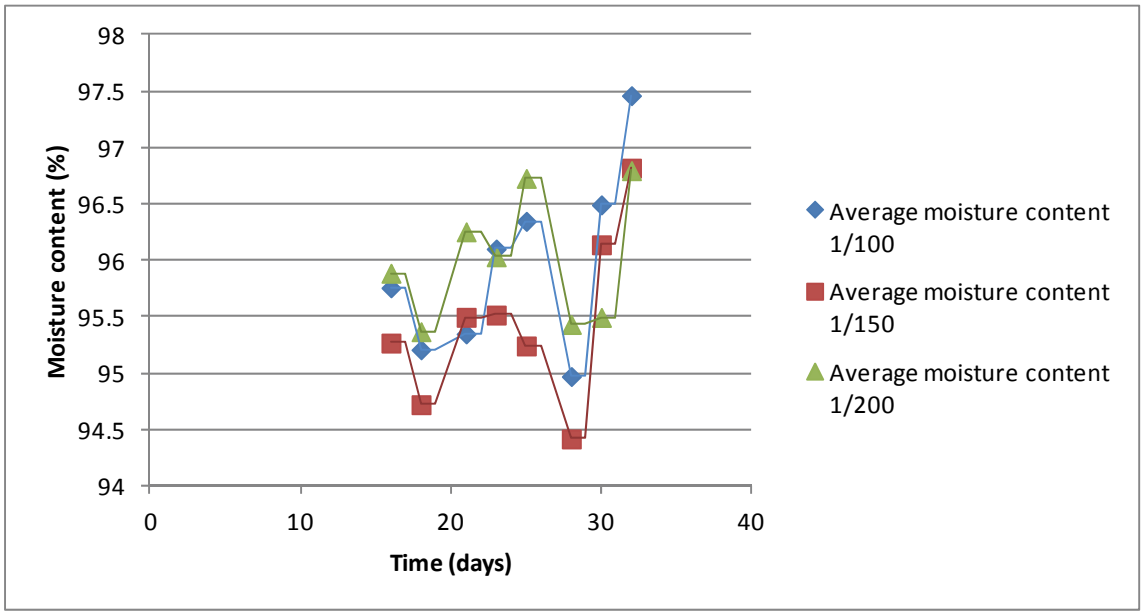

Figure 2-53: Average moisture content of duckweed grown in different Huttner media dilutions at 13°C

The same phenomenon that was noticed in the reactors under controlled conditions was also observed under natural light conditions, where the largest fronds and longest roots were observed at the lower nutrient concentrations.

2.3.2 Nutrient uptake

2.3.2.1 Controlled temperature and light intensity

The uptake of nitrate (NO_3-N) and ortho-phosphorus (PO_4-P) from 1/25 and 1/100 dilutions at different harvesting rates at 25°C is presented in Figure 2-54 and Figure 2-55 respectively. The results indicate that both nitrate and ortho-phosphorus were assimilated by the duckweed in all the experiments during the trials. The ammonia-nitrogen results were below the detection limit within the first three days and are not shown. Nitrate was depleted at a higher rate when compared to ortho-phosphorus.

The uptake of NO_3-N and PO_4-P at different harvesting rates at 18°C and 13°C is illustrated by Figure A-1 to Figure A-8 in Appendix A.

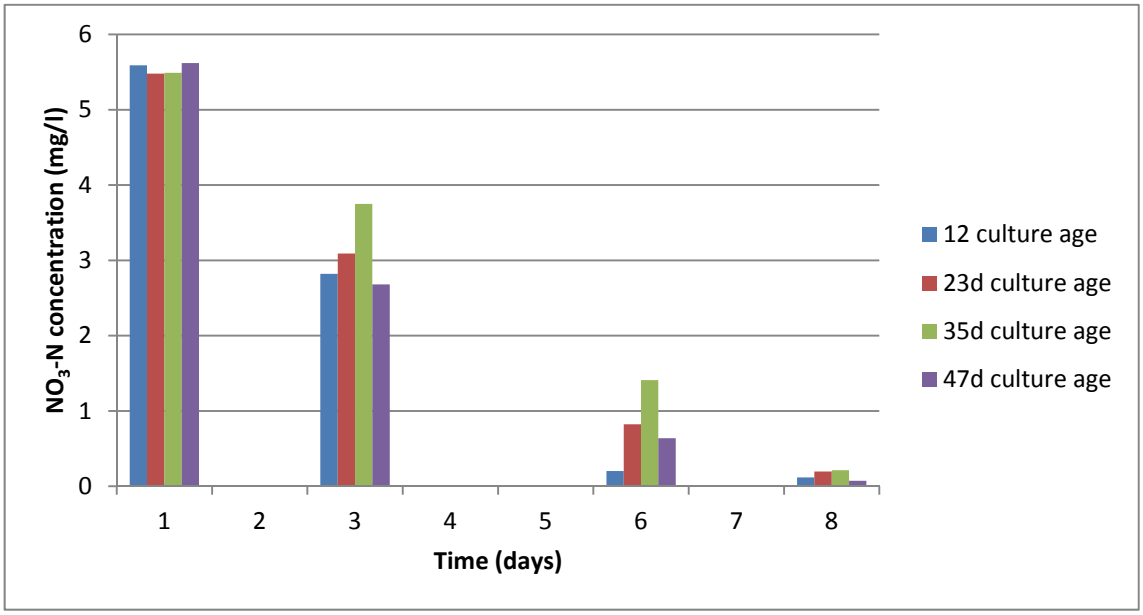

Figure 2-54: Nitrate depletion from 1/25 Huttner media solution at 25°C at different harvesting rates

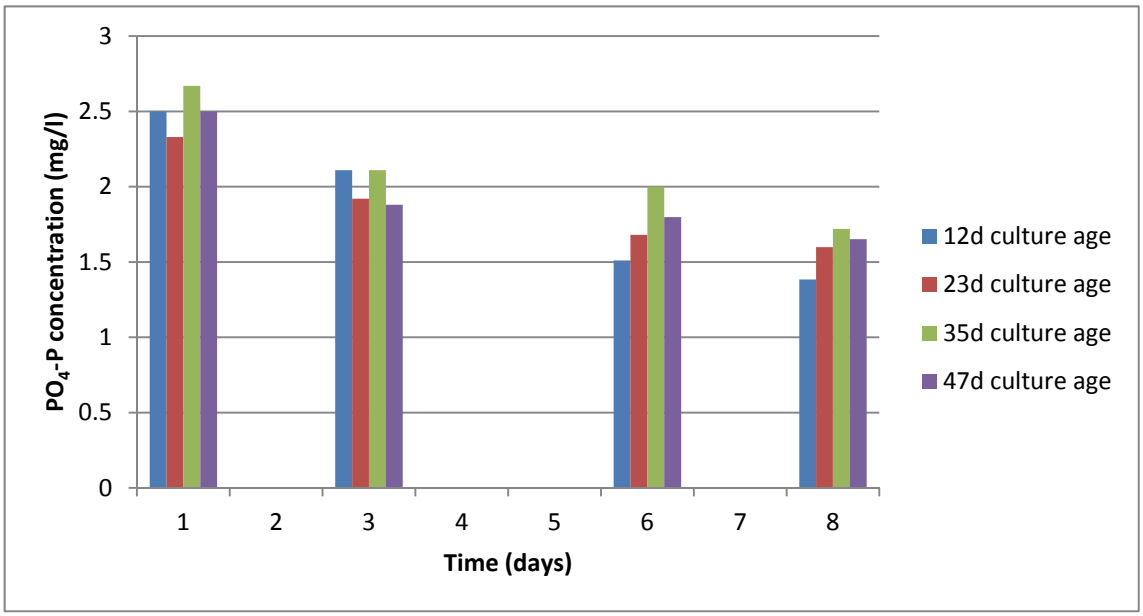

Figure 2-55: Ortho-phosphorus depletion from 1/25 Huttner media solution at 25°C at different harvesting rates

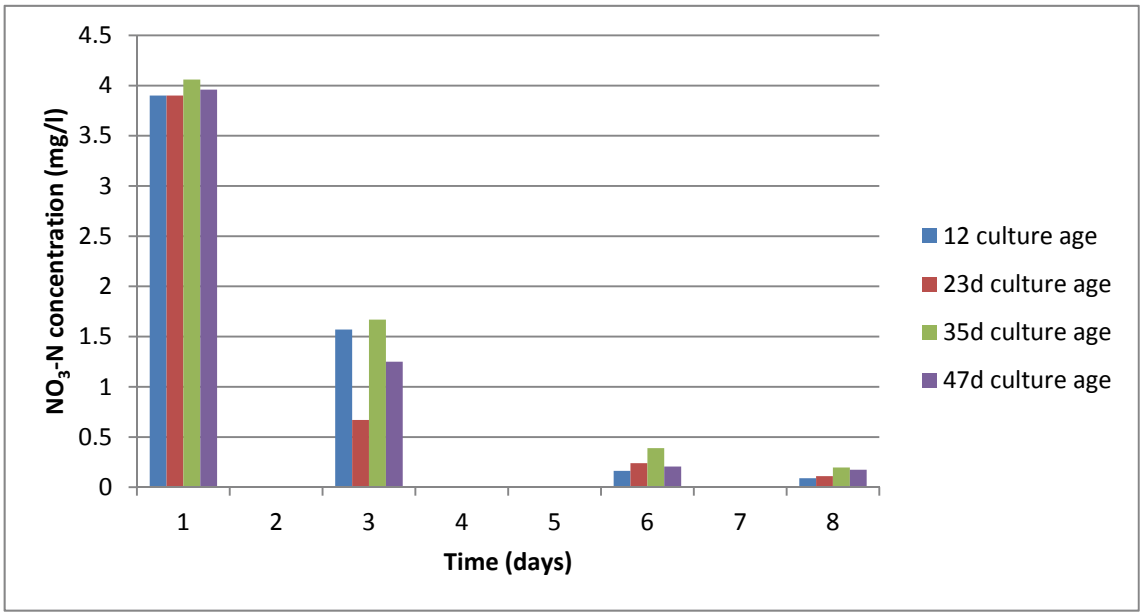

Figure 2-56: Nitrate depletion from 1/100 Huttner media solution at 25°C at different harvesting rates

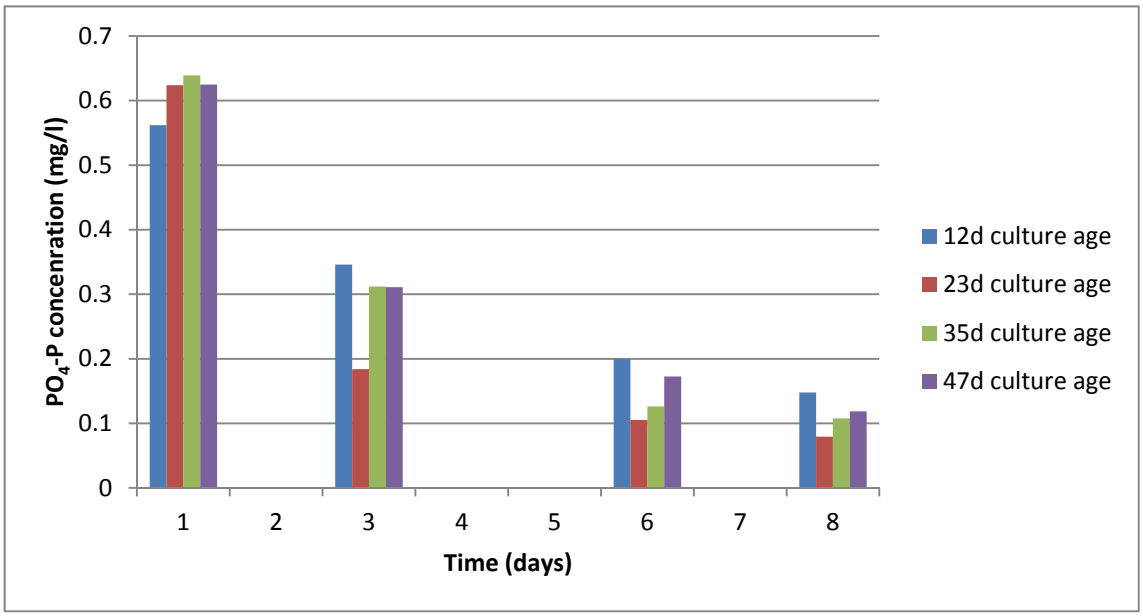

Figure 2-57: Ortho-phosphorus depletion from 1/100 Huttner media solution at 25°C at different harvesting rates

2.3.2.2 Natural light and uncontrolled temperature

2.3.2.2.1 Shade

The average light intensity and temperature were the same as that for the reactor experiments for the period tested.

In the containers harvested to maintain 7d and 12d culture ages, the culture was washed out within 10d. The cultures in the containers harvested to maintain 23d and 35d culture ages maintained a steady surface density (Figure 2-58). The dry mass of duckweed harvested to maintain the cultures is illustrated in Figure 2-59. The uptake of nutrients from solution by the duckweed is demonstrated by the depletion of the nutrients in solution, as illustrated in Figure 2-60 and Figure 2-61. There was a 65% reduction in the ammonia concentration in solution after 15d from 19.2 mg/l to 6.9 mg/l for the 23d culture age, and only a 26% reduction in the nitrate concentration for the same period. For the 35d culture age, the ammonia concentration was reduced by 60% and the nitrate concentration by 21% over the 15d period tested. The 23d culture age therefore demonstrated superior nutrient uptake.

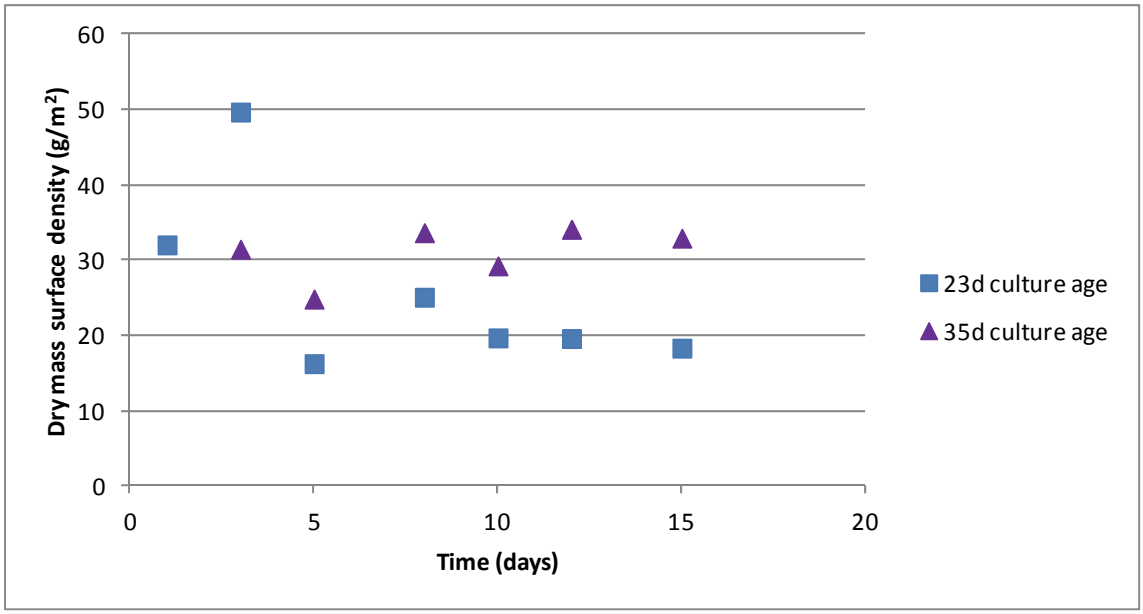

Figure 2-58: Dry mass surface density of containers in the shade harvested to maintain 23d and 35d culture ages

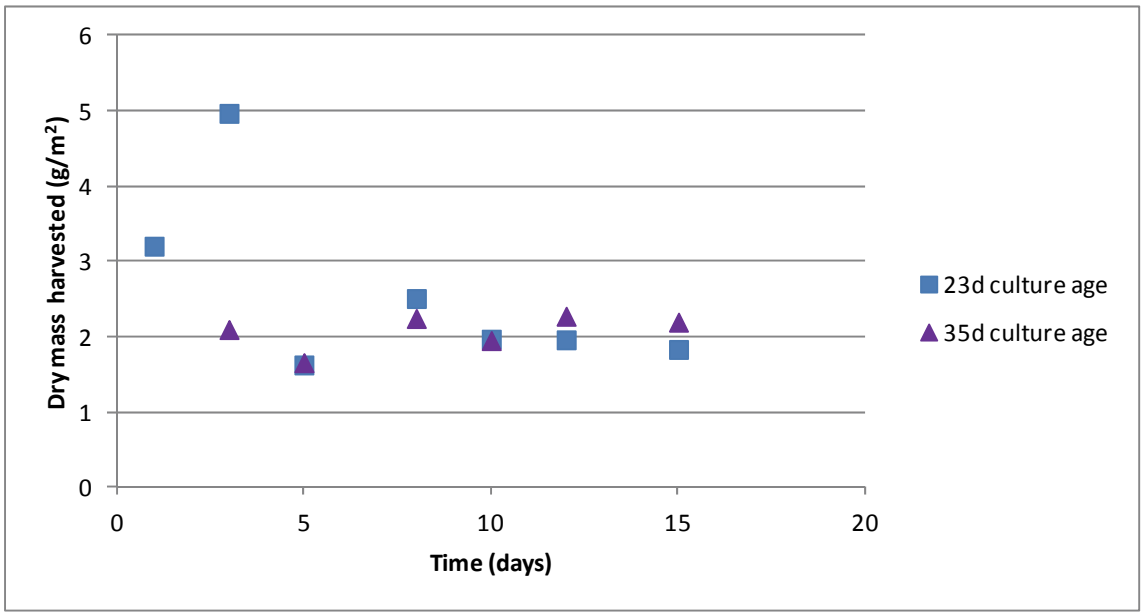

Figure 2-59: Dry mass of duckweed harvested from containers in the shade to maintain 23d and 35d culture ages

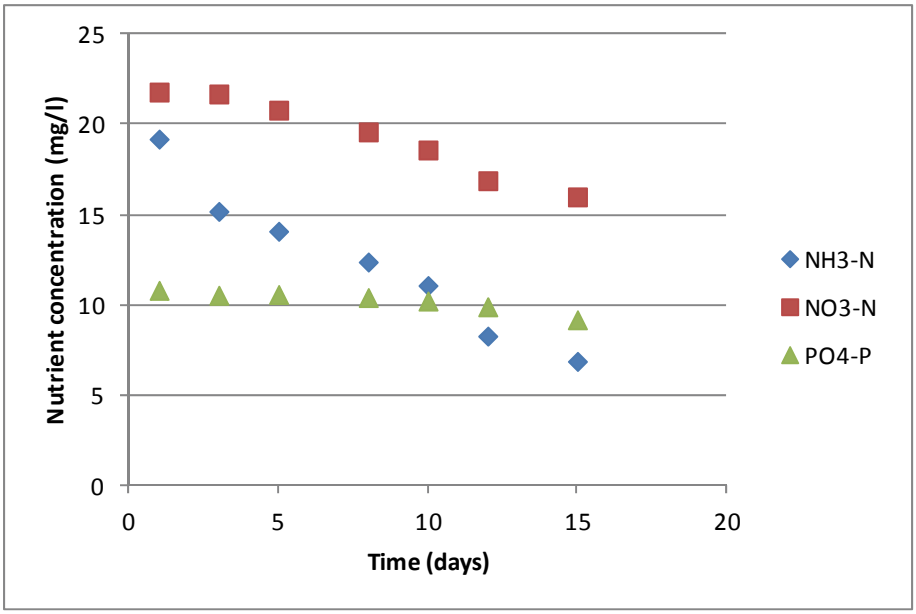

Figure 2-60: Uptake of nutrients from solution in container in shade maintained at 23d culture age

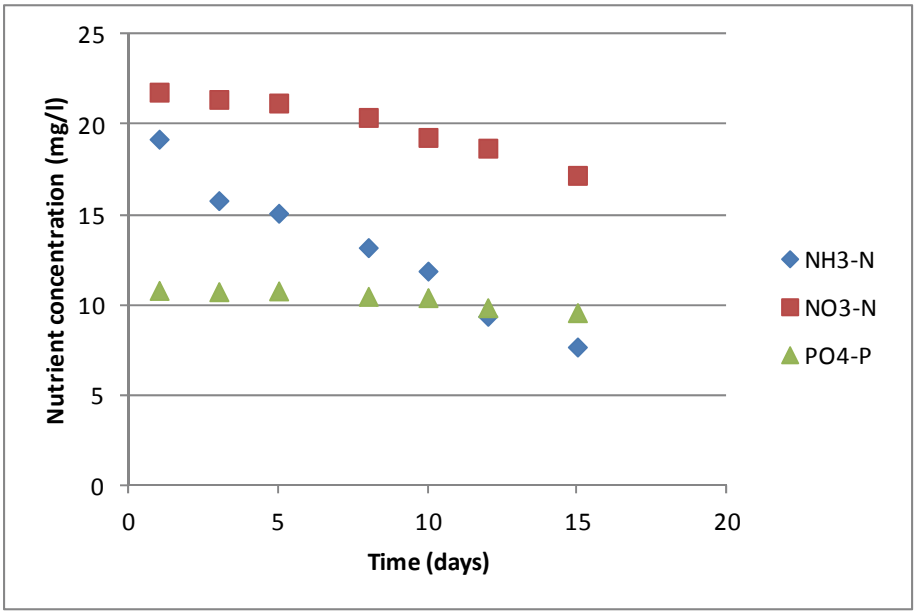

Figure 2-61: Uptake of nutrients from solution in container in shade maintained at 35d culture age

2.3.2.2.2 Sun

The average light intensity and temperature was the same as that for the reactor experiments for the period tested. As observed in the containers in the shade, there was a wash out of the duckweed in the containers maintained at a 7d and 12d culture ages, and the cultures in the containers harvested to maintain 23d and 35d culture ages maintained a steady surface density (Figure 2-62). The dry mass of duckweed harvested to maintain the

cultures is illustrated in Figure 2-63. More effective uptake of nutrients was noted in the 23d culture than in the 35d. Only the results from the 23d culture age are illustrated here (Figure 2-64). The nutrient depletion in the container with the lower nutrient concentration harvested to maintain a 23d culture age is presented in Figure 2-65.

After 15d, there was a 76% removal of ammonia from solution, and a 35% removal of nitrate from the high concentration container (initial NO_3-N and NH_4-N concentrations of 20 mg/l). This was greater than the uptake observed in the shade after the same period of time for the same concentration and harvesting rate (65% for ammonia and 26% for nitrate). After 24d, 99.6% of the ammonia in solution was removed, to a concentration of 0.073 mg/l. 75% of the nitrate was removed during this period. In the low concentration container (initial NO_3-N and NH_4-N concentrations of 20 mg/l), a higher relative uptake rate was observed for ammonia, with a 94% reduction observed within 7d. The nitrate uptake rate was similar, with a 21% reduction in nitrate after 7d.

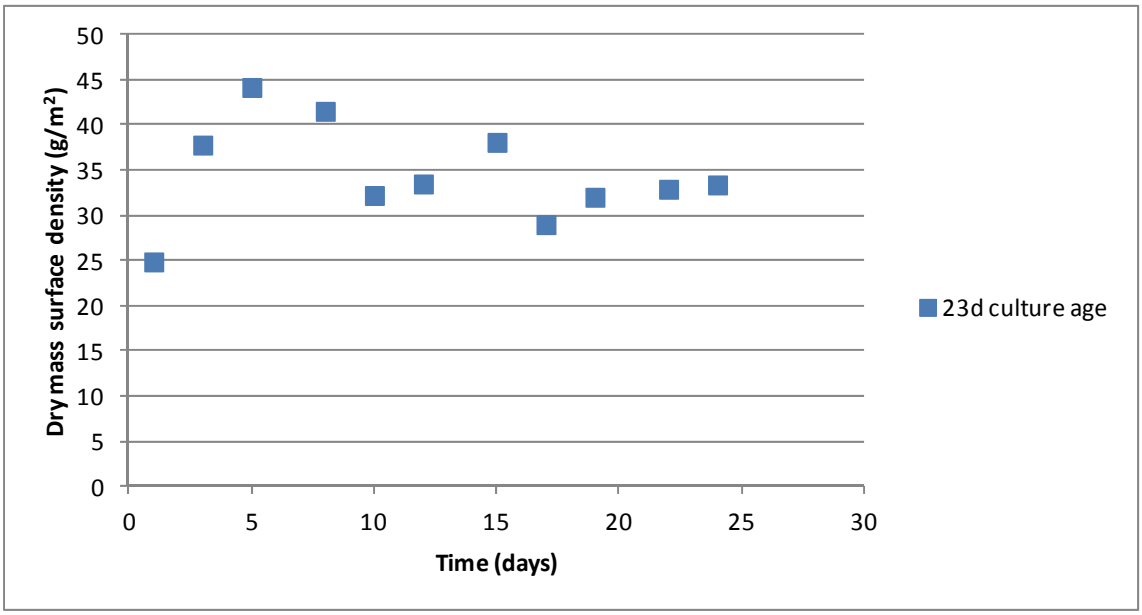

Figure 2-62: Dry mass surface density of containers in the sun harvested to maintain 23d culture age

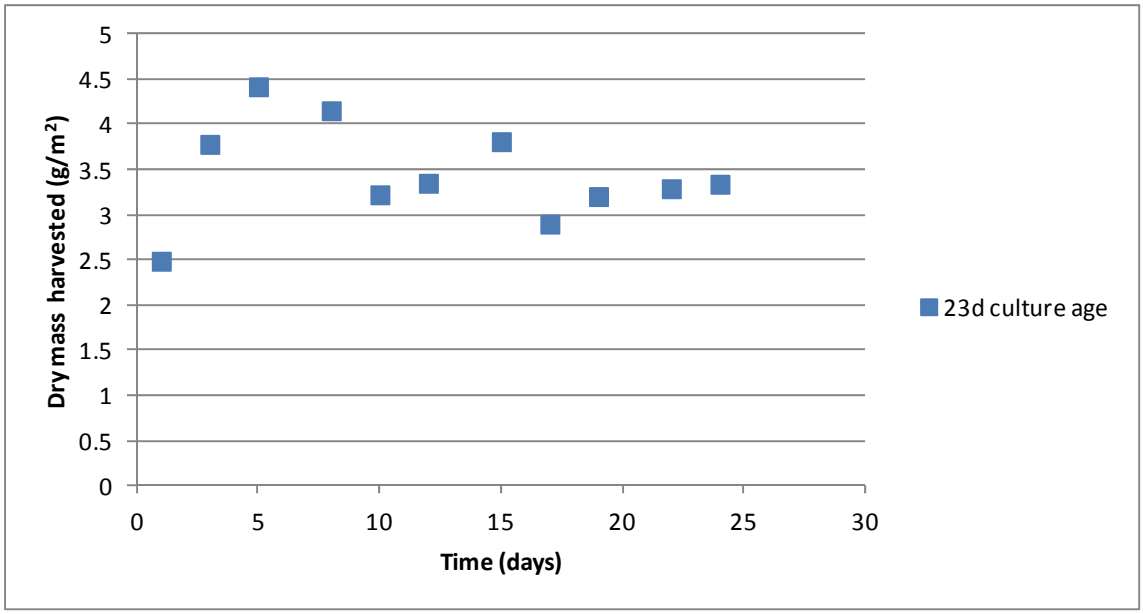

Figure 2-63: Dry mass of duckweed harvested from containers in the sun to maintain 23d culture age

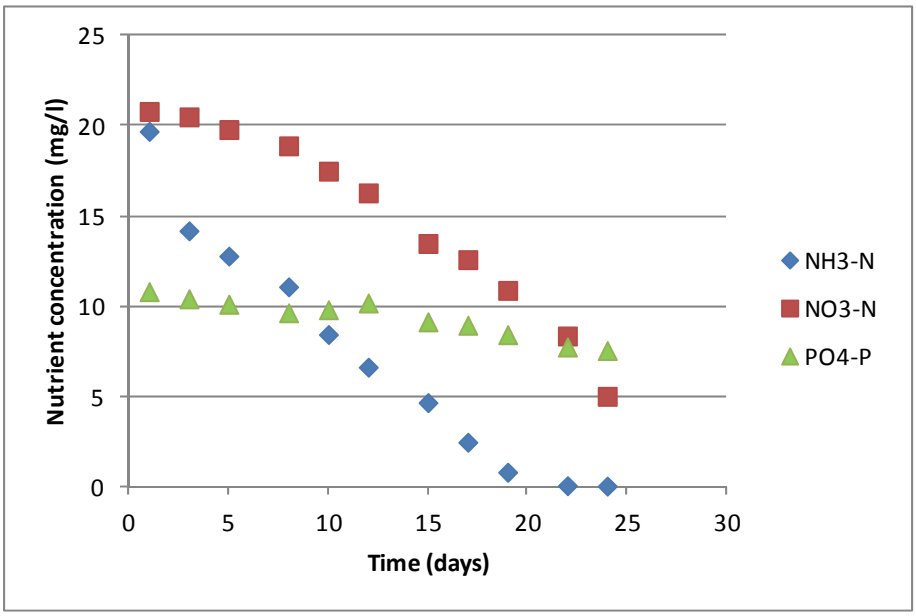

Figure 2-64: Uptake of nutrients from solution in container in sun with high initial nutrient concentration maintained at 23d culture age

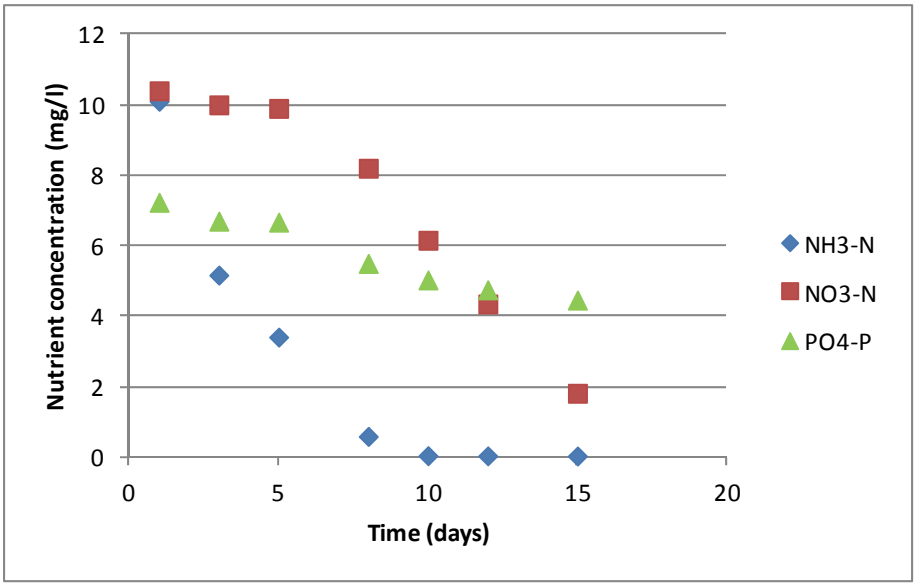

Figure 2-65: Uptake of nutrients from solution in container in sun with lower initial nutrient concentrations maintained at 23d culture age

2.3.3 Dissolved oxygen concentration

The dissolved oxygen (DO) concentration was found to decrease rapidly in the reactors after each nutrient solution replacement. Within three days of nutrient replacement the DO concentration decreased to below 0.2 mg/l at the bottom of the reactors at all temperatures, indicating anaerobic conditions. The dissolved oxygen concentrations observed in the 25°C reactor for the 1/5, 1/25 and 1/100 nutrient concentrations are presented in Figure 2-66 to Figure 2-68. After the light had been on for at least 8 hours, higher dissolved oxygen concentrations of between 0.2 and 0.6 mg/l were observed below the duckweed layer than were measured at the bottom of the reactor.

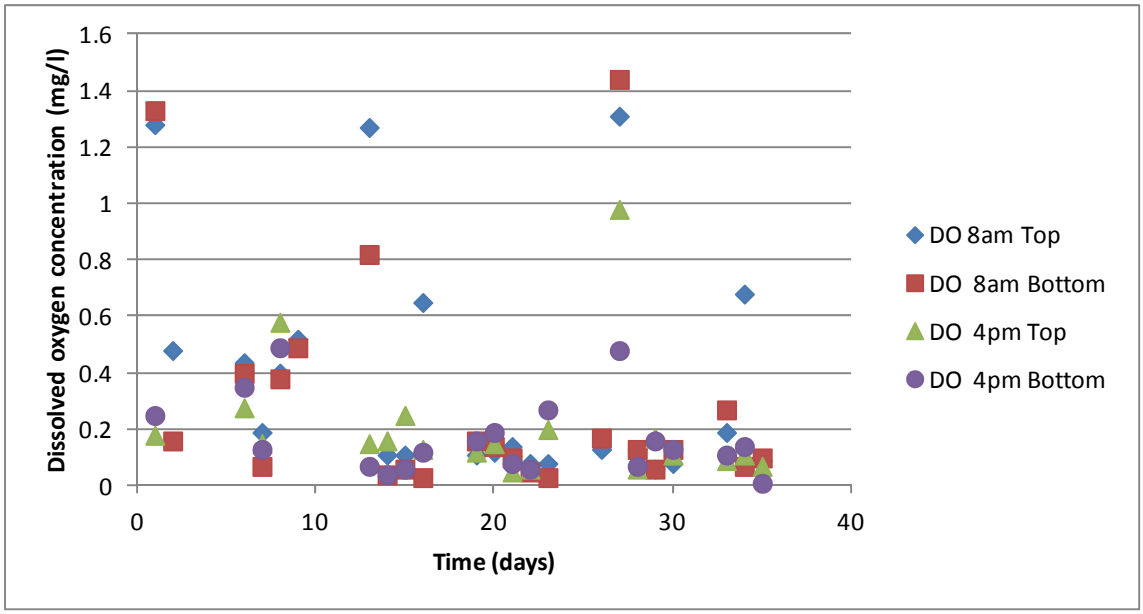

Figure 2-66: Dissolved oxygen concentration in chamber with 1/5 Huttner media at 25°C

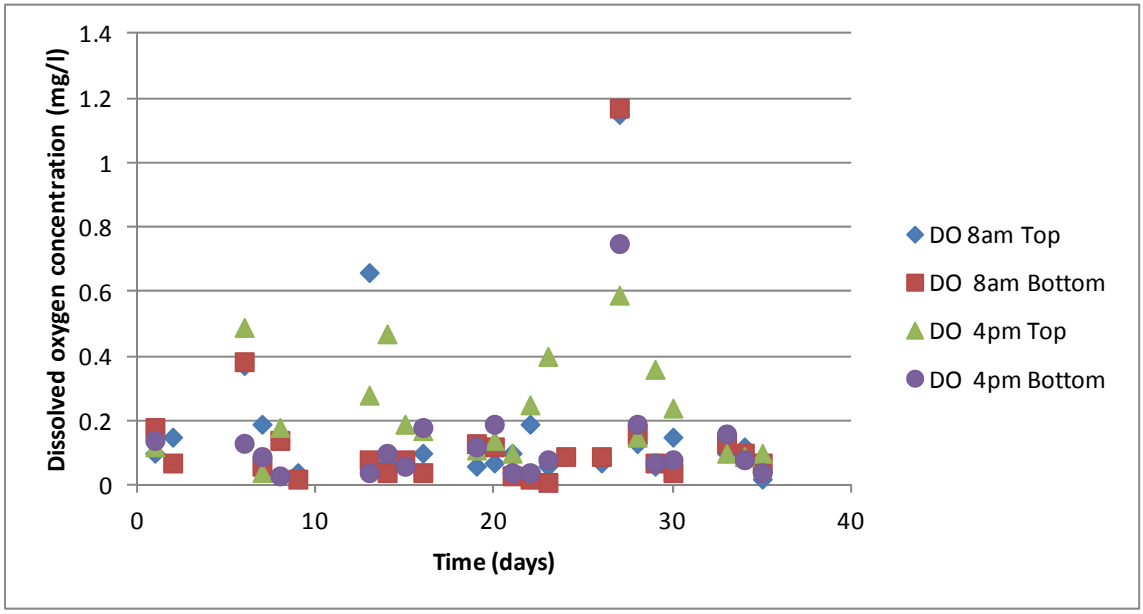

Figure 2-67: Dissolved oxygen concentration in chamber with 1/25 Huttner media at 25°C

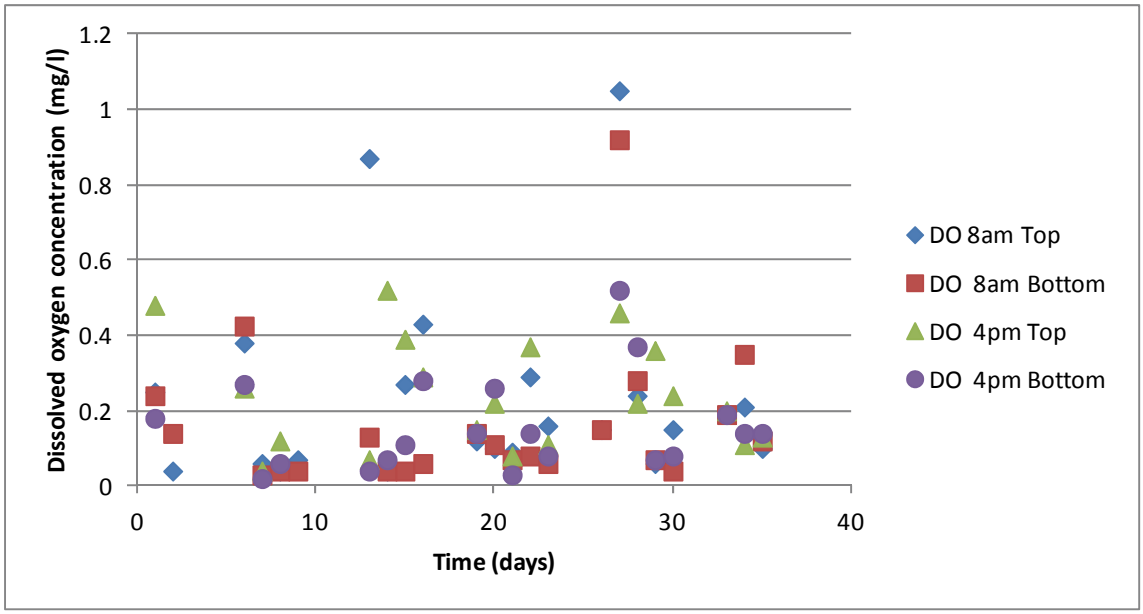

Figure 2-68: Dissolved oxygen concentration in chamber with 1/100 Huttner media at 25°C

2.4 Discussion

2.4.1 Theoretical duckweed growth model

Duckweed reproduce sexually or vegetatively. Duckweed are monocotyledons and bear stamens and carpels on the same flower. It is therefore possible to reproduce through self-pollination, although insects and wind may assist with pollination. The observed rapid growth of duckweed, however, is the result of vegetative reproduction. Only vegetative growth has been observed during this study and fieldwork and therefore formed the basis of this study.

Most species of duckweed have two birth spots from which daughter fronds are emerging during the lifetime of an individual plant. While a daughter plant is emerging from one birthspot, the next daughter is developing in the second birth spot. The duckweed species differ in the level of maturity reached by the daughter fronds before being released from the mother. A plant is considered mature when the first daughter emerges. In some species, the daughter fronds are released from the mother before the daughter reaches maturity. This implies that the daughter frond will develop further after being released and then start reproducing. These species are characterised by either single plants or a mother plant with an emerging daughter plant; therefore no more than two plants are attached to each other. In other species, the daughter frond is still attached the mother frond after reaching maturity. It implies that the original frond may carry two daughter fronds, while the daughter fronds also carry emerging second-generation fronds. In this way, small colonies are formed containing a number of generations. In species where the frond has only one birth spot, it is possible to observe a short chain of individual plants attached to each other. Since a daughter frond must be released from the mother frond before the next daughter can develop at the same birth spot, the maximum number of individuals in a colony is 7 (with two birth spots), while the maximum the maximum number of individuals in a chain is 3 (with only one birth spots). Daughter fronds are produced at regular intervals. The period between the intervals and the number of daughters produced during the lifetime of an individual may vary and are determined by the species and environmental conditions. Since the duckweed found in a specific environment is genetically homogenous, the variation between individuals is limited and the number of intervals and the period between intervals in a community may be treated as constants. The mathematical model is based on this principle. In order to illustrate the concept, an example is given of a frond with two birth spots and with the ability to produce three siblings during its life-time (Figure 2-69). When a control volume is inoculated with an immature individual, the first daughter emerges after one period. The second daughter emerges after the second interval while the first daughter

reaches maturity with its first daughter just emerging. The colony consists of 4 individuals after the second interval. By the end of the third interval, the first daughter of the original frond is released carrying a mature daughter (already with an emerging frond) and an immature daughter (forming a colony of 4 individuals), while the original frond carries the matured second daughter (with an emerging frond) and an immature third daughter. The original frond releases the third (final) daughter and dies. By the end of the fourth interval, therefore, the control volume contains the dead original frond, all three daughter fronds and their siblings. The total number of dead plants in the culture is one while it contains 14 viable plants at this stage. The first daughter dies after the fourth interval, while more siblings emerge from the viable plants, implying two dead plants in the culture after the fifth interval, while the number of viable plants equals 26. This clearly implies an exponential growth curve.

Using the same arguments for fronds with the ability to produce two or more daughters per frond, it is possible to predict the number of siblings produced per frond after a certain number of intervals, if the culture is reproducing exponentially. This is illustrated in (Table 2-10).

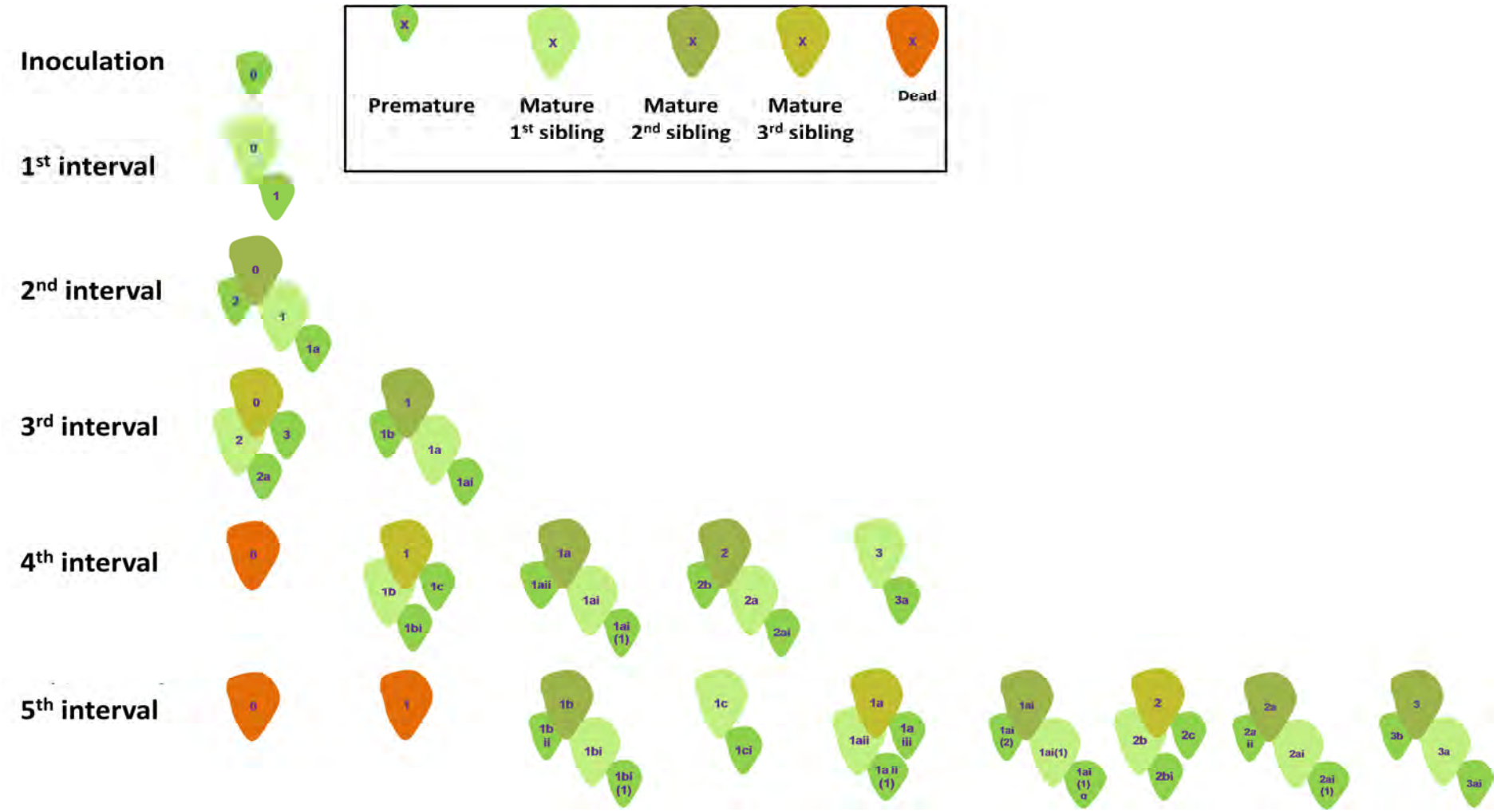

Figure 2-69: Duckweed growth model: Example of parents producing 3 siblings during life-cycle

Table 2-10: Duckweed growth to illustrate effect of number of siblings per parent on expected population size

No of birth intervals	No. of siblings														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2
3	1	3	4	4	4	4	4	4	4	4	4	4	4	4	4
4	1	5	7	8	8	8	8	8	8	8	8	8	8	8	8
5	1	8	13	15	16	16	16	16	16	16	16	16	16	16	16
6	1	13	24	29	31	32	32	32	32	32	32	32	32	32	32
7	1	21	44	56	61	63	64	64	64	64	64	64	64	64	64
8	1	34	81	108	120	125	127	128	128	128	128	128	128	128	128
9	1	55	149	208	236	248	253	255	256	256	256	256	256	256	256
10	1	89	274	401	464	492	504	509	511	512	512	512	512	512	512
11	1	144	504	773	912	976	1004	1016	1021	1023	1024	1024	1024	1024	1024
12	1	233	927	1490	1793	1936	2000	2028	2040	2045	2047	2048	2048	2048	2048
13	1	377	1705	2872	3525	3840	3984	4048	4076	4088	4093	4095	4096	4096	4096
14	1	610	3136	5536	6930	7617	7936	8080	8144	8172	8184	8189	8191	8192	8192
15	1	987	5768	10671	13624	15109	15808	16128	16272	16336	16364	16376	16381	16383	16383

2.4.2 Effect of nutrient composition and harvesting regime at different temperatures and light intensities

2.4.2.1 Growth rate

A mass balance is required to model the growth of duckweed on the surface of the reactors. For convenience, the model is based on dry mass of duckweed per unit area. The control area of the model is the surface area of a single reactor. Generally, a mass balance for the duckweed growth is written based on the mass of duckweed entering and leaving the reactor, being generated and being consumed. The mass balance therefore takes the form:

Nett rate of accumulation on the reactor surface
= rate of flow into the control area
– rate of flow out of control area
– rate of harvesting
+ nett rate of generation on the control surface area

or simply

$$\text{Accumulation} = \text{input - output} + \text{generation} \qquad (2.3)$$

It is assumed that the biomass was uniformly distributed over the entire surface of the reactor and that the walls of the reactor confined the boundaries of the reactor. The terms in the mass balance have the units of mass/day. Duckweed was regularly harvested from the surface by isolating a fixed fraction, n, of the total surface area and removing all the duckweed from the isolated area. Gentle mixing of the surface area after harvesting ensured that the biomass remained uniformly distributed. It is further assumed that there was no input of duckweed into the reactor with the feed, and no loss of duckweed with the effluent. Therefore, the input term equals zero, except for the initial inoculation, while the output equals the rate of harvesting. Consequently, equation 2.3 can be simplified as follows:

$$\text{Accumulation} = \text{harvesting} + \text{generation} \qquad (2.4)$$

Let A be the area of the reactor expressed as m^2, X the dry biomass density measured as $g.m^{-2}$, t the time expressed as days, r_h and r_g respectively the harvesting and growth rates measured as $g.m^{-2}.day^{-1}$, then equation 2.4 can be expressed as follows:

$$A\frac{dX}{dt} = -Ar_hX + Ar_gX \qquad (2.5)$$

The harvesting rate is expressed as a fraction of the biomass removed per day and is equal to n. The population age, Θ, is equal to the inverse of the harvesting rate. Therefore:

$$r_h = n = \frac{1}{\Theta} \tag{2.6}$$

Inserting this relationship in equation 2.5 and dividing by A, gives the following relationship:

$$\frac{dX}{dt} = -\frac{1}{\Theta}X + r_g X \tag{2.7}$$

Separating the variables and integrating equation 2.7 gives the function of biomass density with time, considering that X_0 is the biomass density at the time, t_0, when the measurements are first recorded:

$$\ln\frac{X_t}{X_0} = \left(-\frac{1}{\Theta} + r_g\right)(t_t - t_0) \tag{2.8}$$

or

$$\ln X_t = \ln X_0 + \left(r_g - \frac{1}{\Theta}\right)(\Delta t) \tag{2.9}$$

where $\Delta t = t_t - t_0$.

Taking the antilog of both sides of equation 2.9, then:

$$X_t = X_0 e^{\left(r_g - \frac{1}{\Theta}\right)\Delta t} \tag{2.10}$$

Three scenarios can be considered. When the generation rate exceeds the harvesting rate, duckweed will accumulate on the surface. The derivative in equation 2.7 will be positive. When the generation rate equals the harvesting rate, there will be no accumulation and the population will be at steady state. The derivative in equation 2.7 will equal zero. Finally, when the generation rate is less than the harvesting rate, the population density will decline and eventually be eliminated from the reactor. The derivative in equation 2.7 will be negative. Each scenario will be demonstrated below, using arbitrarily selected data sets from the experiments.

The data from the experiment at 25°C and a culture age, Θ, of 12 days is presented in Figure 2-70 to illustrate the behavior when $r_g > 1/\Theta$.

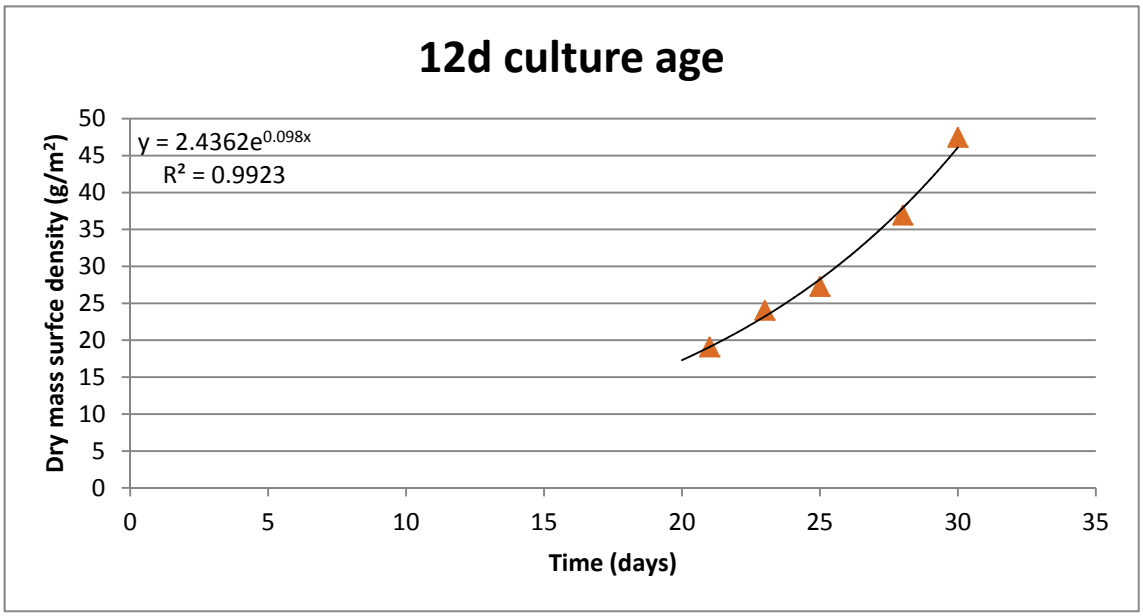

Figure 2-70: Demonstration of experimental data where the growth rate exceeds the harvest rate

The best-fit curve gives the following relationship:

$$X_t = 2.44e^{0.098t}$$

Therefore:

$$r_g - \frac{1}{\theta} = 0.098$$

$$r_g = 0.098 + \frac{1}{\theta}$$

$$r_g = 0.098 + \frac{1}{12} = 0.098 + 0.083 = 0.181 d^{-1}$$

Washout will occur when more than 18% of the duckweed biomass is removed daily. Since only 8.3% has been removed daily, the biomass accumulated on the surface of the reactor. The excellent fit of the data with an exponential function indicates that the growth of the duckweed was unlimited and first-order.

In order to illustrate the scenario where $r_g = 1/\Theta$, the data for the reactor operated at 18°C with 1/200th dilution of the Huttner solution and 35 day culture age was selected (Figure 2-71).

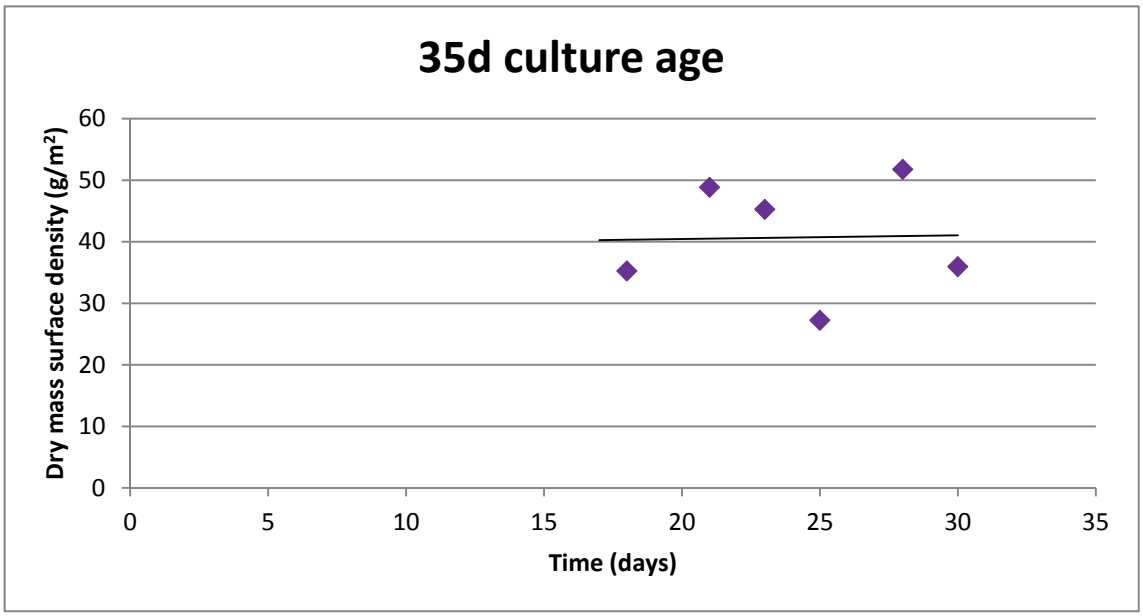

Figure 2-71: Demonstration with experimental data where the growth rate equals the harvest rate

The biomass density remained constant at 41 g/m^2, therefore:

$$r_g - \frac{1}{\theta} = 0$$

or

$$r_g = \frac{1}{\theta} = \frac{1}{35} = 0.029 d^{-1}$$

The growth rate is low and the population will be washed out when it is harvested at a rate higher than 2.9% per day.

Where the harvest rate exceeds the growth rate, the duckweed population declined until it was completely washed out. The data from the culture grown at 18°C in a 1/25th Huttner solution and with Θ of 23 days illustrate the scenario (Figure 2-72). One data point, clearly an outlier on the 19th day was removed from the data set.

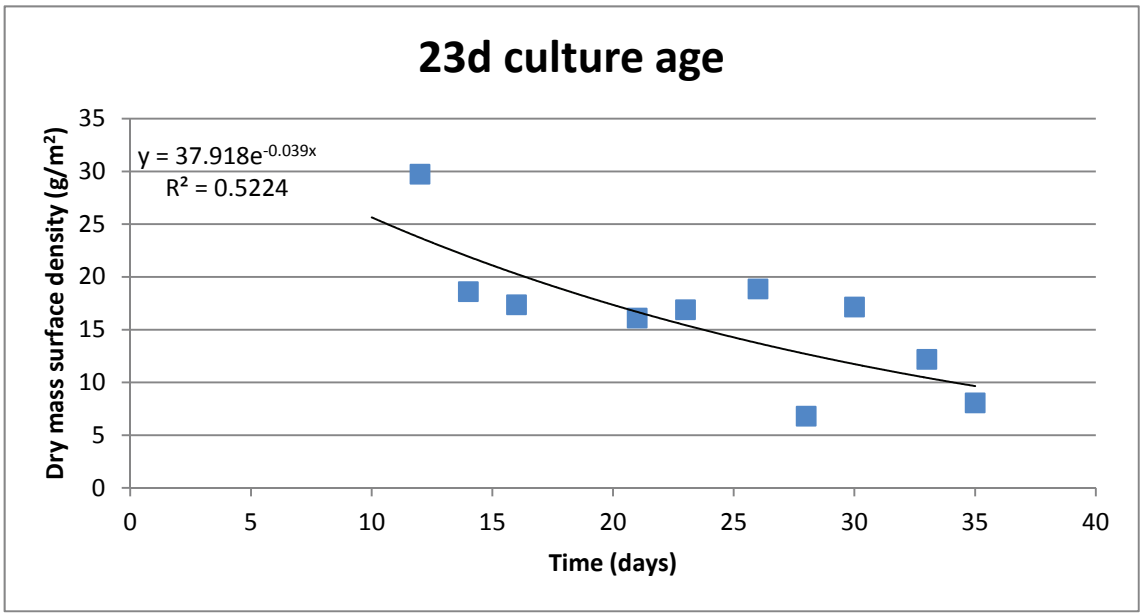

Figure 2-72: Demonstration with experimental data where the growth rate equals the harvest rate

The best-fit curve gives the following relationship:

$$\ln X_t = 37.918e^{-0.039t}$$

Therefore:

$$r_g = -0.039 + \frac{1}{\theta} = -0.039 + \frac{1}{23} = 0.004 \text{ d}^{-1}$$

In this example, it can be seen that the growth rate is very small, but positive. Washout of the culture can only be prevented if the harvesting rate is reduced to less than 0.4% per day, which is equivalent to 250 days.

Finally, an example can be illustrated where the harvest rate exceeds the growth rate and the duckweed population declines until it is completely washed out and where the results indicate the presence of a toxic substance. The data from the culture grown at 25°C in a 1/5th Huttner solution and with Θ equals 23 days have been used to demonstrate this (Figure 2-73).

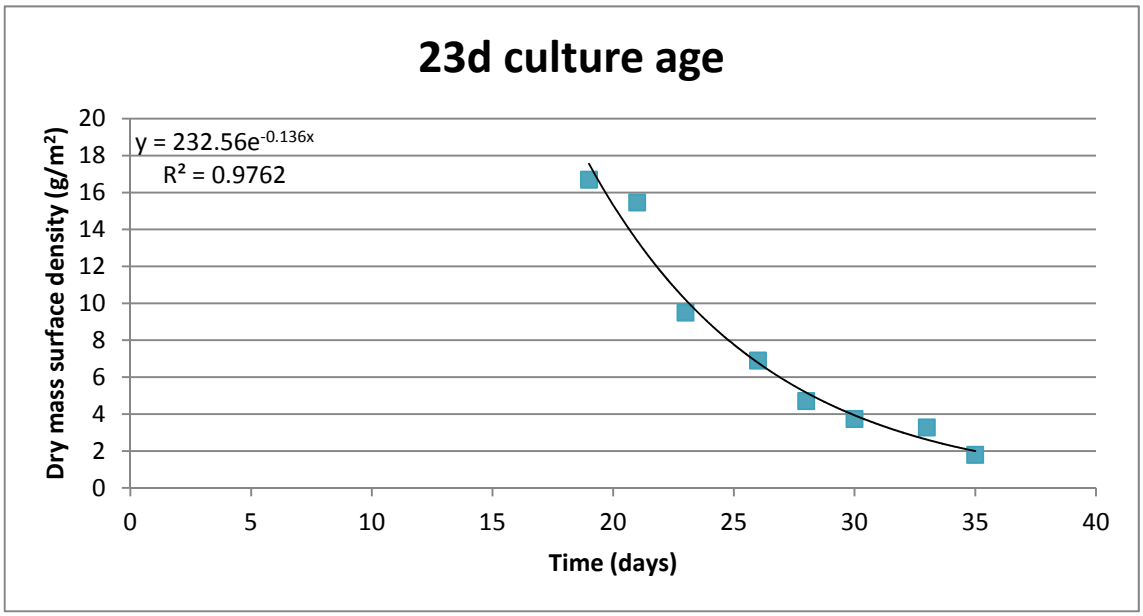

Figure 2-73: Demonstration with experimental data where the growth rate equals the harvest rate and the presence of a toxic substance.

The best-fit curve gives the following relationship:

$$\ln X_t = 232.56e^{-0.136t}$$

Therefore:

$$r_g = -0.136 + \frac{1}{\theta} = -0.136 + \frac{1}{23} = -0.093\ d^{-1}$$

In this example, the growth rate is negative; implicating exposure to a toxic substance. The culture died at a rate of 9.3% per day and could not survive even if harvesting ceased.

This method has been used for all the experimental data after equilibrium was established. Best-curve exponential lines were fitted to each data set for all the experiments, even if they were not a good fit. The purpose was to table the intrinsic growth rates for all the treatments. This information was used in an attempt to investigate the effects of substrate concentration, temperature and light intensity on the intrinsic growth rate. The coefficient of equation 2.9 is equal to r_g-1/Θ and is easily calculated considering that it is a linear function. Using the method of regression analysis, the coefficient for each experiment was determined. The probable range of the coefficients for each experiment was determined by applying an analysis of variance (ANOVA) at a 95% confidence level. The error bars in the figures are equal to ± 1 standard deviation.

The results are shown in Figure 2-74 to Figure 2-78. Note that the experiments were repeated twice with a 1/100th dilution of the Huttner media with different harvesting ranges at 13 and 18°C.

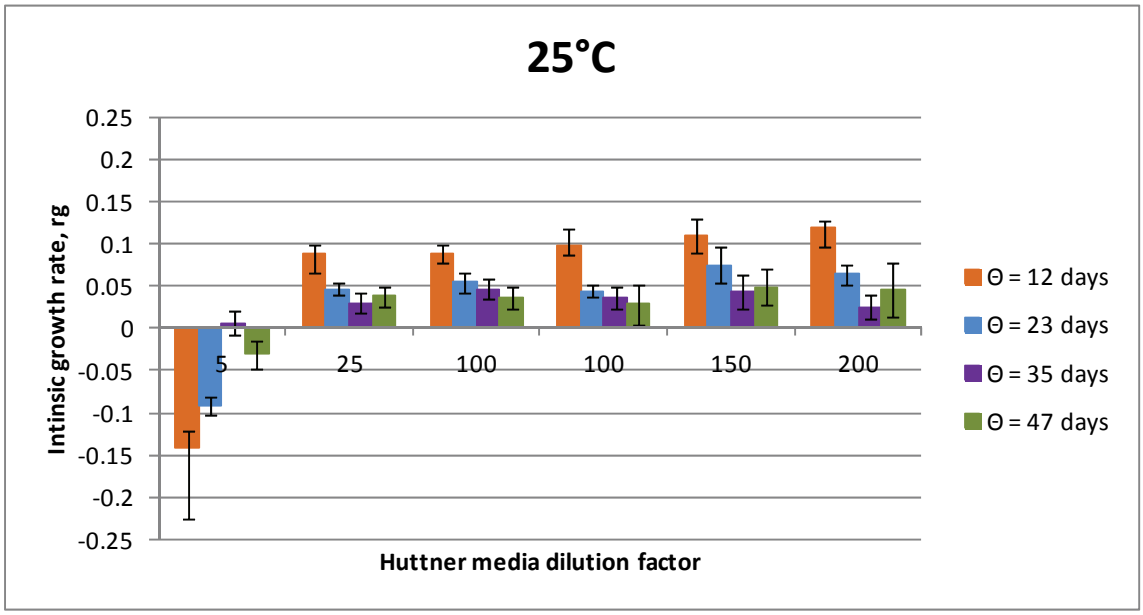

Figure 2-74: Intrinsic growth rate measured at varying harvesting rates and dilutions of Huttner media at 25°C

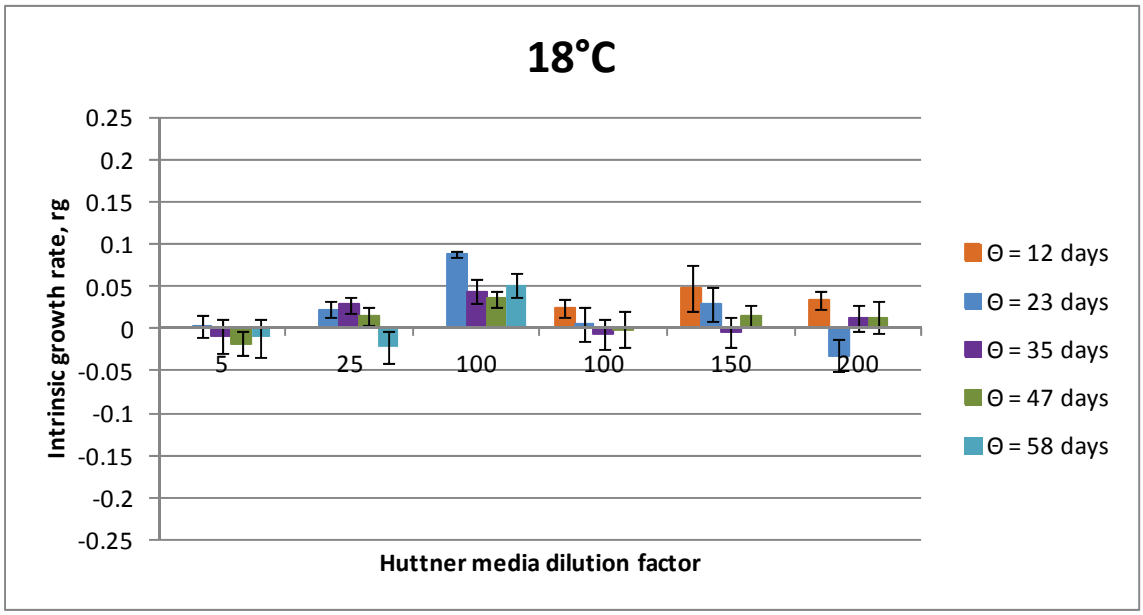

Figure 2-75: Intrinsic growth rate measured at varying harvesting rates and dilutions of Huttner media at 18°C

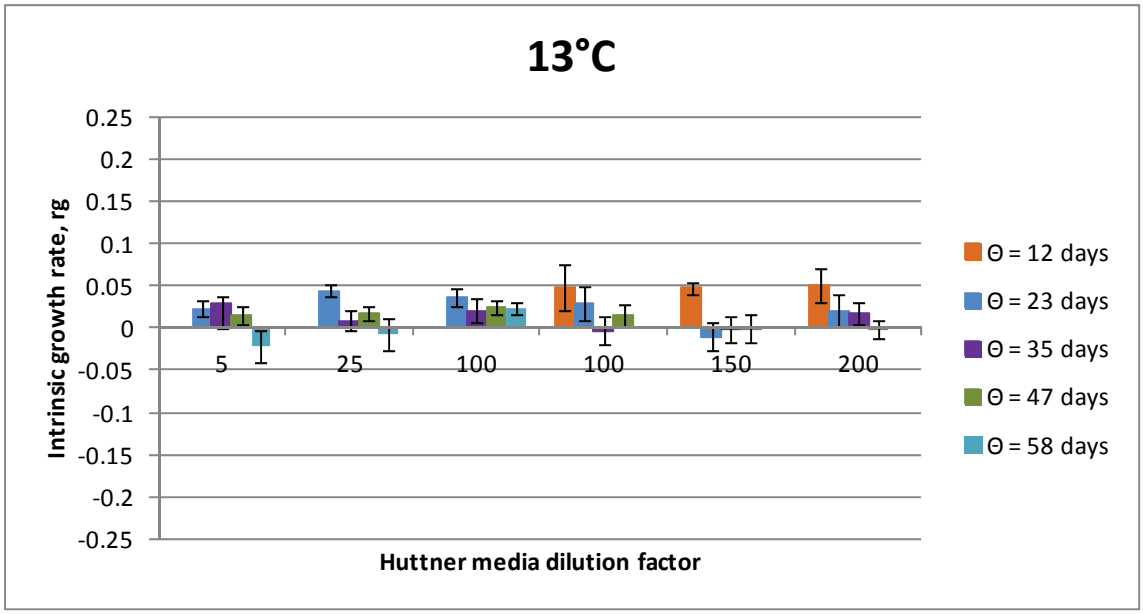

Figure 2-76: Intrinsic growth rate measured at varying harvesting rates and dilutions of Huttner media at 13°C

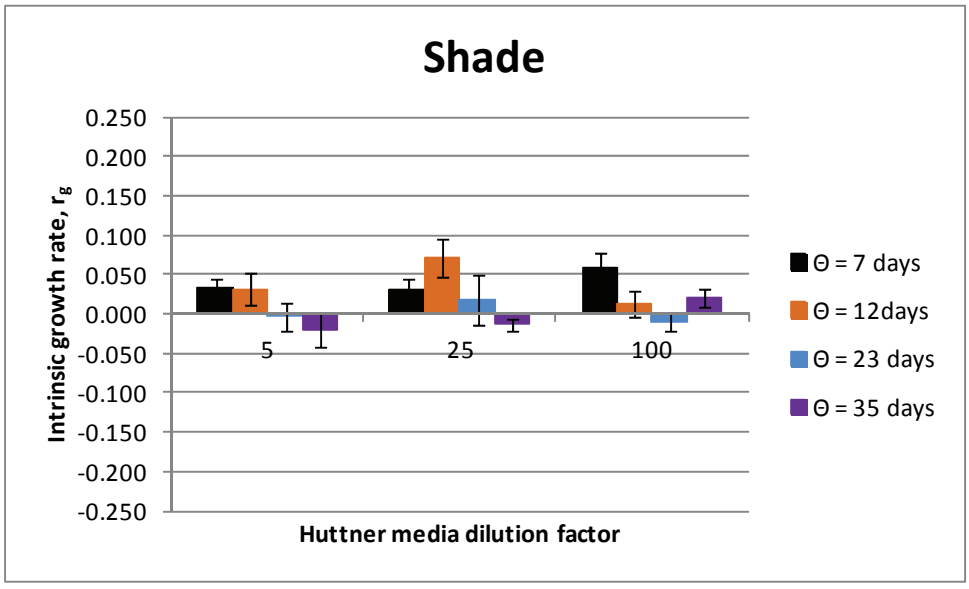

Figure 2-77: Intrinsic growth rate measured at varying harvesting rates and dilutions of Huttner media in the shade without temperature control (average temperature 24.8°C, average mid day light intensity 8325.3lux)

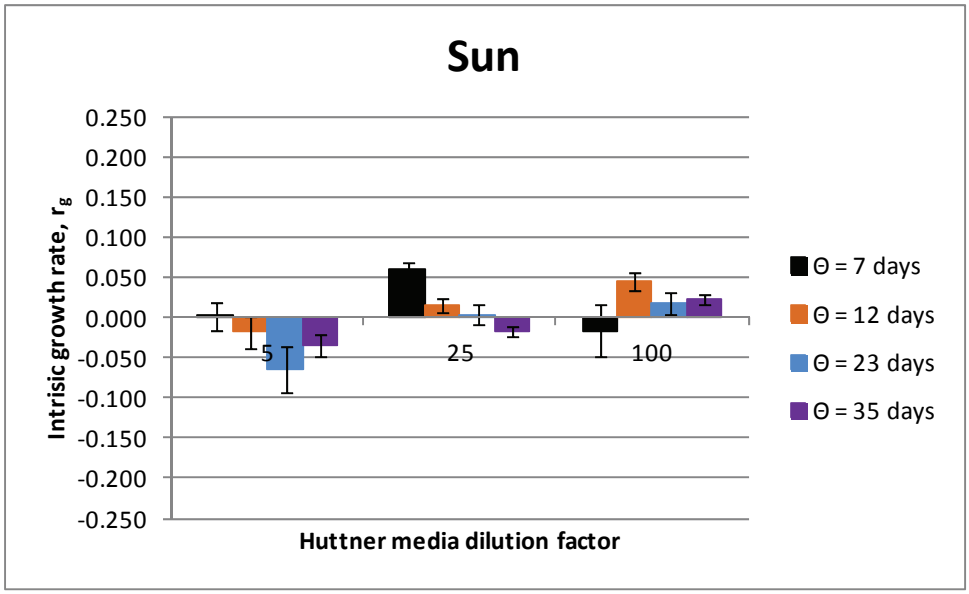

Figure 2-78: Intrinsic growth rate measured at varying harvesting rates and dilutions of Huttner media in the sun without temperature control (average temperature 27.8°C, average mid day light intensity 86234.7lux)

The following observations were noted with respect to the intrinsic growth rate:

- The growth rates in all the experiments under controlled conditions were negative at a concentrated Huttner solution (1/5th dilution), implying toxicity of the substrate or stimulation of senescence.
- The growth rates at all other dilutions and harvesting rates under controlled conditions were positive at 25°C, with a general increase at lower concentrations (higher dilution factors)
- The growth rate at the short culture age (12 days) was generally higher compared with the longer culture ages under controlled conditions at 25°C, implying that the biomass surface density had an effect (negative) on the growth of the duckweed. It is an indication that the cultures are light limited and that the shading effect at higher mat densities under the experimental conditions may be significant. The dry mass surface density of the 12 day culture age was generally lower compared with the longer culture ages (see Figure 2-6, Figure 2-8, Figure 2-10 and Figure 2-12). Körner & Vermaat (1998) and Chaiprapat et al. (2005) observed a similar reduction in growth rate and nutrient uptake in high density duckweed cultures, where the higher density limited access to nutrients by the duckweed located in the upper layers, and limited light, gas exchange, and space to grow reduced the potential for nutrient uptake. Monette et al. (2006) found a maximal biomass growth rate of 88 g-dry.m^{-2} (1470 g-wet.m^{-2}) at an optimal initial mat density of 45 g-dry.m^{-2} (750 g-wetm^{-2}), with

removal rates for nitrogen (N) and phosphorus (P) of 483 mg-N.m^{-2}.d^{-1} and 128 mg-P.m^{-2}.d^{-1}, respectively.

- Despite all the obstructions to growth and nutrient uptake, higher surface density does offer some benefits. A dense layer prevents light penetration to the liquid medium and thus inhibits algal growth. It could also help lower the ammonia emission to the atmosphere by providing shading to the pond water that keeps the water temperature and pH from strong diurnal changes, such as commonly observed in conventional stabilization ponds (Zimmo et al., 2003), and creating a lower pH layer near the water surface as a result of nutrient uptake from the water by duckweed (Chaiprapat et al., 2003). Finally, the overall duckweed biomass production can be higher at relatively high density even with a lowered specific growth rate. A higher total number of fronds (in high density) to reproduce would at some point balance out and surpass the overall growth of the lower-density culture with a higher specific growth rate.
- The growth rates at 13°C and 18°C were generally slightly positive or negative and randomly distributed around zero-growth, indicating that duckweed ceased to grow at low temperatures. The observation that nutrients were assimilated, even when the growth ceased, indicated that the duckweed were not dying, but rather accumulating and storing products inside the plants.
- At the nutrient concentrations tested in the shade, the intrinsic growth rates were higher than those observed under controlled conditions at 25°C for 12, 23 and 35d culture ages. With the exception of the 23d culture age at the 1/100 dilution, positive growth rates were observed for all conditions tested. This was expected due to the higher light intensity. The shorter 7d culture age that was tested here showed a higher growth rate in the 1/5 and 1/25th dilutions when compared with the longer culture ages. This supports the theory that shading affects the growth rate of the culture by limiting the accessibility of light to the plants. It is therefore important that the duckweed is harvested to maintain a thin mat to achieve the optimal growth rate.
- There was not an obvious increase in the growth rate of the culture grown in the sunlight when compared to that grown in the shade. Negative growth rates were observed in the 23d culture at 1/5th dilution, and the 7d culture at the 1/100 dilution, resulting in a wash out of duckweed. There is clearly no benefit to having a light intensity that exceeds the saturation light intensity for duckweed of approximately 18500lux.

2.4.2.2 Biomass composition and nutrient storage

The results of the two-way ANOVA analysis indicated that the concentration of nutrients in the media did not have an effect on the composition of the biomass, at least at the

concentrations tested. Landolt & Kandeler (1987) reported that nitrogen and phosphorus concentrations in constant nutrient solution higher than about 4 mg/L did not raise the biomass nitrogen and phosphorus contents of duckweed further.

In reactors under controlled conditions of light intensity and temperature, the temperature applied had a significant effect on the TKN and total phosphorus concentrations of the biomass, with the highest concentrations observed in the 25°C reactor. Temperature did not affect the COD concentration of the biomass however, as there was no significant difference between the biomass grown at different temperatures. In contrast, light intensity had a significant effect on the COD composition of the plants, with the highest COD concentration observed in the plants grown in sunlight, but had no significant effect on the concentration of TKN and total phosphorus in the biomass. A higher light intensity therefore appeared to increase the rate of photosynthesis, thus increasing the rate of carbohydrate synthesis in the plant, expressed as a higher COD concentration per kg of biomass.

The highest average biomass nitrogen and phosphorus contents observed were 5.6% (56 $g_{TKN}/kg_{biomass}$) and 1.27% (56 $g_{TP}/kg_{biomass}$) respectively from the reactor in the sun, and the lowest were 4.2% (42 $g_{TKN}/kg_{biomass}$) and 0.6% (6 $g_{TP}/kg_{biomass}$) from the reactor at 13°C. This is comparable to the minimum and maximum nutrient concentrations observed for the duckweed *Spirodela punctata* by Chaiprapat et al. (2005). The authors found that the difference between the maximum and minimum biomass contents could be stored in the duckweed biomass and possibly used for its growth. A higher rate of uptake was noted near the beginning of the experiments, when nutrient accumulation took place, indicating that starving duckweed could take up nutrients at a higher rate to “fill up” their storage capacity. When the storage was full, intake of nutrients was purely controlled by growth rate. The results of this study indicate that temperature affects the capacity of the ability of the plants to store nutrients.

This is an important operational consideration of a duckweed system, as the dry mass of duckweed harvested will not necessarily indicate the amount of nutrients removed in all conditions. There will be a minimum nutrient concentration in the biomass when the duckweed plants are starved of nutrients, but if conditions are favorable for storage then the nutrient removal rate per dry mass of duckweed will be much higher. At high light intensities the amount of biomass is expected to be higher, but the nutrient concentration will not necessarily follow the same trend, as the nutrient uptake is temperature dependant. Where the temperature is low, it will be necessary to increase the surface area of duckweed to increase the capacity of the system for nutrient removal, due to the lower rate of accumulation at lower temperatures.

2.4.2.3 Species composition

2.4.2.3.1 Controlled conditions of temperature and light intensity

Within a few days, all the *Lemna gibba* plants disappeared from the stock culture, as well as from the experimental populations. Because the nutrient concentrations and temperatures remained constant, and the *Wolffia* and *L. turionifera* populations continued to thrive, this was most likely as a result of a greater sensitivity of *Lemna gibba* to the sub optimal light intensity of approximately 4000 lux (Lasfar et al., 2007). The *Wolffia* plants were less tolerant to low temperatures than the *L. turionifera* plants, as they were rapidly washed out at 13°C and 18°C in all concentrations of Huttner media. At 25°C, the *L. turionifera* plants dominated the cultures at all concentrations with the exception of the 1/5 dilution, where the *Wolffia* plants were more numerous. This indicated that the *Wolffia* were more tolerant to the high nutrient concentration than the *L. turionifera* at 25°C. Temperature therefore plays an important role in the species composition that can be expected in a natural system.

2.4.2.3.2 Natural light and uncontrolled temperature conditions

Although a similar temperature was observed in the reactor in the shade (24.8°C) as the controlled temperature in the 25°C room, a difference was noticed in terms of the species composition. Within 1 week *Wolffia* plants became dominant in all Huttner media dilutions in the shade, whereas these plants were only dominant in the temperature controlled room in the 1/5 dilution. The higher light intensity therefore played a role in the dominance of the *Wolffia* plants.

Lemna turionifera became dominant in all concentrations tested in the sun. The temperature in the reactor in the sun was 27.8°C on average, and the light intensity often exceeded that of photo inhibition. The *Lemna turionifera* plants therefore dominated under conditions of both high temperature and high light intensity.

2.4.2.4 Plant physiology and nutrient uptake

The nutrient concentration affected both the root length and frond size of the *L. turionifera* plants, with the root length increasing with decreasing nutrient concentrations. The frond sizes increased with decreasing nutrient concentrations at 25°C only, with reduced frond sizes being observed at both 18°C and 13°C. There was a decrease in the moisture content of the plants grown at 25°C in the 1/100, 150 and 1/200 dilutions, but this was not observed at 18°C and 13°C, where the moisture constant remained constant and increased respectively. The larger plant fronds observed at lower nutrient concentrations were therefore more dense than those grown at the lower temperatures. This reiterates the observation above

that the duckweed growth rate increases at low concentrations at 25°C. The growth inhibiting effect of the high nutrient concentration appeared to be exacerbated by the light limiting conditions.

Turions, or winter buds, are specially produced, dense, starchy, frondshaped bodies by which many species of duckweed overwinter in temperate zones. The turions are produced vegetatively in the budding pouch(es) of the parent frond. Turions have reduced air spaces and contain many starch grains which enable them to sink, thus avoiding extreme temperature fluctuations. Germination of the turions occurs under conditions favorable for vegetative growth of the fronds (Dudley, 1987).

Turion formation was noted in the 1/25 Huttner dilution at 18°C. Henssen (1954) found that cultures of *Spirodela polyrrhiza* formed turions under moderate nutrient and mineral deficiencies, with maximum formation during the winter months. Hillman (1961) reported that turions were produced under any condition which would maintain photosynthesis at levels considerably in excess of carbohydrate utilization for growth and respiration. Thus turions occurred even at relatively low light intensities when growth was reduced by nitrogen deficiency. Newton et al. (1978) found that increased levels of NO_3^- inhibited turion production in both the presence and absence of sucrose, but there was also a reduction in frond number; this was especially apparent when the medium was deficient in Ca^{2+}. It was concluded that increased levels of NO_3^- along with additional amounts of Ca^{2+} and sucrose stimulated turion production, and that the stimulation was preceded by increased frond proliferation. Dudley (1987) studied turion formation in *Lemna minor* and *L. turionifera*, and observed that nutrient limitation, especially of phosphorus and/or nitrogen, did not appear to be the principal cause of turion induction, but that the addition of sucrose encouraged turion formation and phosphorus addition encouraged turion germination. In the experiments conducted, no sucrose or other carbon source was added to the Huttner media tested, and the light intensities were growth limiting. Turion formation was observed only at a moderately limiting nutrient concentration and the moderately low temperature of 18°C. No turion formation was observed in either the 25°C or 13°C experiments. This is in agreement with the observations of Henssen (1954) and Hillman (1961). The formation of turions in duckweed applied to water treatment systems should be avoided, as there will be no net population growth, with plants channelling their energy into starch storage. The culture should not be allowed to become nutrient limited, especially at colder temperatures and high light intensities.

Lehman et al. (1981) found that the protein content in the *Lemna* fronds is higher than the protein content of the roots, while nitrate content in the roots is much higher than in the

fronds. The enrichment of protein in leaves and nitrate in roots is characteristic of monocotyledons such as barley and is thought to be related to higher levels of nitrate reductase in leaves. Despite the fact that the fronds float on the nutrient medium and all cells are within a few cell lengths of the nitrate supply, this tissue distinction is also found in duckweeds. Since nitrate reductase is rapidly substrate-induced in Lemnaceae, one might expect the nitrate pool size to remain constant. The authors observed that both protein and nitrate accumulated in larger quantities and in higher concentrations at 23.9°C than at 18.3°C. They proposed two mechanisms to explain this; raising the incubation temperature could cause an increase in the rate of nitrate accumulation over nitrate reduction to produce the larger nitrate and protein pool sizes. A second mechanism which could contribute at least part of the increases in nitrate and protein is a possible decrease in size of the amino acid pool which lies in the chemical pathway between nitrate reduction and protein formation. When *Lemna* growth is reduced (by incubation without nutrients), protein degradation rates are increased while protein formation rates decline. The slower growth rate recorded at 18.3°C might be accompanied by an increase in the pool size of free amino acids at the expense of the nitrate precursor and protein products.

Plants, algae, and all photosynthesizing organisms use the nitrogen from ammonia, not nitrates, to produce their proteins. If the plant takes up nitrate, it must first be converted to ammonium through nitrate reduction. Nitrate reduction in plants appears to be the mirror image of the bacterial process of nitrification. Nitrifying bacteria gain the energy they need for their life processes solely from oxidizing ammonium to nitrates; the total energy gain from the two-steps of nitrification is 84 Kcal/mol. Plants theoretically must expend essentially the same amount of energy (83 Kcal/mol) to convert nitrates back to ammonium in the two-step process of nitrate reduction. The energy required for nitrate reduction is equivalent to 23.4% of the energy obtained from glucose combustion (Hageman, 1980). Thus, if nitrifying bacteria in biological systems such as algal ponds convert all available ammonium to nitrates, aquatic plants such as duckweed will be forced, at an energy cost, to convert all the nitrates back to ammonium. Porath & Pollock (1982) evaluated the duckweed *Lemna gibba* L. for its potential as a biological ammonia stripper. Ammonia uptake was compared with respect to varying conditions of circulating water, temperature, pH, and nitrate concentrations. Results indicated that uptake is an active process with preference for ammonia over nitrate. In an axenic culture of 0.1--0.3% duckweed biomass, *Lemna gibba* stripped 50% of the ammonia present at levels 10^{-4} M $NH_3 \leftrightarrow NH_4^+$ in 5h, while the nitrate level (10^{-2} M NO_3^-) remained constant. Alaerts et al. (1996) assessed the performance of a full scale duckweed-covered sewage lagoon, and based on their findings the authors suggested that microbial hydrolysis of complex organic N and P into NH_4^+ and ortho-PO_4^{3-} was the limiting step for enhanced duckweed biomass production, as the intensive

harvesting (every 2-3d) reduced the possibility that nitrifying bacteria (or cyanobacteria fixing atmospheric nitrogen) would thrive on the plants' surface or root zone. It was concluded that adequate pre-treatment for the sewage should be provided to release organically bound NH_4^+ and ortho-PO_4^{3-}.

The nutrient uptake trials conducted in this study demonstrate this phenomenon, with the ammonia concentration being reduced preferentially at all temperatures under controlled conditions. Although the nitrate uptake rate was less rapid than that of ammonia, which was reduced to below detection in solution within 3 days, there was consistently high uptake with the concentrations being reduced from 6 mg/l to below 1 mg/l NO_3-N within 8 days. Phosphorus uptake was also observed at all nutrient concentrations and temperatures. It is important to note that even when the cultures appeared to have a negative growth rate, such as was observed at 13°C, there was still nutrient assimilation, albeit at a slower rate than at the higher temperatures of 18°C and 25°C. When the effect of higher initial concentrations of nutrients on the uptake rate was tested in the shade and the sun, the 23d culture age showed the highest nutrient uptake rate. A higher uptake rate was observed for ammonia over the same relative time period when the initial concentration was 10 mg/l (94%) than when the initial concentration was 20 mg/l (76%). As was observed under controlled conditions, ammonia was taken up preferentially to nitrate.

From these results and the information available in the literature, it is clear that the nitrogen in waste water should not be allowed to be converted to nitrate before assimilation by the duckweed, as this expends unnecessary energy. It is therefore recommended that the duckweed pond(s) immediately follow the anaerobic pond in the treatment series, to optimise the nitrogen uptake rate. High concentrations of ammonia may limit the initial rate of uptake, so there may be merit in diluting the effluent entering the duckweed ponds with treated water through recycle, either of the final effluent or of the effluent of the duckweed pond(s).

2.4.3 Importance of mixing

The reactors used in this study were designed to ensure that the nutrient supply to the duckweed surface layer was not diffusion limited, by creating completely mixed conditions. Monselise & Kost (1993) observed a faster rate of ammonium-ion absorption in their stirred duckweed culture flasks compared to the unstirred. They suggested that depleting local $NH4^+$ ions near the plant as a result of its nutrient uptake caused the reduced uptake. Al-Nozaily et al. (2000) reported that mixing had a significant positive effect on their duckweed system.

Nitrogen removal by duckweed in a static pond depends on the combined action of ammonium transport, which conveys the ions to the surface, and duckweed ammonium uptake at the surface. Experimental results reported by Chaiprapat et al. (2003) showed a lower ammonium ion concentration near the surface of a static duckweed reactor. The authors concluded that a low concentration of nutrients near the surface was unfavorable to the nutrient recovery and removal, and that ammonium transport was the limiting step in the process.

Design of duckweed ponds should be planned to avoid transport limited conditions. Uptake-limited conditions will occur in well-mixed systems where the productivity of duckweed and the nutrient removal efficiency are at maximum, i.e. when the mixing intensity is below the adversity level that stresses the duckweed. In such a system with regular duckweed harvesting, the removal of nutrients will be directly governed by the uptake and growth characteristics of the duckweed applied, and may be species specific.

2.4.4 COD removal in duckweed-based systems

After the light had been on for at least 8 hours, higher dissolved oxygen concentrations of between 0.2 and 0.6 mg/l were observed below the duckweed layer than were measured at the bottom of the reactor, indicating limited oxygen diffusion into the water as a result of duckweed photorespiration. After an 8h dark period, this oxygen was once again depleted to below 0.2 mg/l. Alaerts et al. (1996) reported that a full scale duckweed pond system had a fairly constant high DO of 2-4 mg O_2/l along the whole length of the pond, which suggested adequate re-aeration. The authors suggested that duckweed pond systems removed organic matter primarily through aerobic heterotrophic oxidation, requiring active diffusion or transportation of oxygen into the liquid phase. Similarly, Körner et al. (1998) reported that degradation of organic material was enhanced by duckweed through both additional oxygen supply and additional surface for bacterial growth. The results of the reactor experiments reported here contradict these results. As was shown in the tracer study, the reactors were not diffusion limited, but within a 3 day period the dissolved oxygen concentration was depleted in the reactors to below 0.2 mg/l, even though no source of COD was added to the growth media. It is therefore clear that anaerobic COD removal is important in duckweed-based systems. This in is agreement with the results reported by Al-Nozaily et al. (2000), who investigated the effect of depth, mixing intensity and sewage concentration on the COD removal, DO levels and the pH of duckweed sewage lagoons. The authors found that removal of COD did not differ between duckweed-covered and control reactors, and the role of duckweed cover was marginal in changing the redox potential or the DO concentration. COD removal correlated strongly with initial surface load. Concentration removal was also proportional to initial COD concentration.

2.4.4.1.1 Role of sulphate reduction under anaerobic conditions

It has been observed in duckweed-based systems in the Limpopo Province that the ponds remain anaerobic, with low DO concentrations of less than 0.3 mg/l measured at the outlet of each pond, including the discharge. Elemental sulphur, an oxidation product of hydrogen sulphide, has been observed to precipitate on surfaces of the overflow weirs between ponds (Figure 2-79). The presence of sulphate in the inflow of sewage encourages the growth of sulphur reducing bacteria under the anaerobic conditions of the ponds. These bacteria play an important role in the uptake and removal of COD from the system, resulting in the reduction of sulphate to sulphur as was observed in the ponds. The mechanism of COD removal in duckweed ponds is therefore unlikely to be as a result of the growth of aerobic heterotrophic bacteria, as was indicated by Alaerts et al. (1996) and Körner et al. (1998).

Figure 2-79: Elemental sulphur, an oxidation product of hydrogen sulphide, precipitates on the overflow weirs of duckweed-based maturation ponds

2.5 The PETRO process; application and lessons learned

2.5.1 Description of the PETRO process

PETRO is an acronym for Pond Enhanced TReatment and Operation. PETRO is an integrated pond system, which employs both stabilization ponds as a primary stage and a conventional process, either activated sludge reactor or trickling filters, as a secondary stage (Shipin et al., 1997; 1999 a.b). These unit processes, arranged in a specific way, result in a synergistic treatment effect. Recirculation is an indispensable property of the system. The basic flow diagram is shown in Figure 2-80.

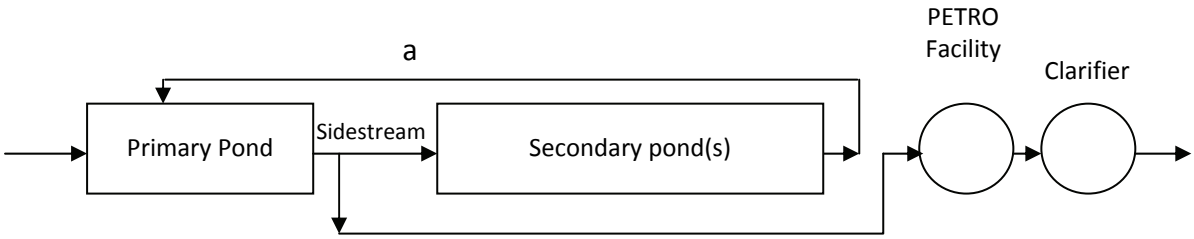

Figure 2-80: Flow diagram of the PETRO system. A: algae-rich recycle; PETRO facility is either an activated sludge reactor or trickling filter (Shipin & Meiring, 1997)

2.5.1.1 Primary ponds

In the primary pond, up to 60% of COD is removed (Meiring and Hoffmann, 1994). Also of importance is the production of an effluent that contains relatively high levels of readily biodegradable matter, such as volatile fatty acids. This is achieved by sidestream recirculation of oxygen rich algae-containing water from the secondary ponds. This oxygenated water partially suppresses the growth of anaerobic methanogenic bacteria, which otherwise would deplete the volatile fatty acids and other readily biodegradable matter in the effluent passing out of the primary pond.

These organic compounds provide energy to the mechanism that removes microalgae by means of entrainment/autoflocculation in the downstream PETRO reactor. If correctly employed, the compounds can also facilitate biological phosphorus removal in the downstream PETRO reactor. Both procedures require a supply of readily biodegradable matter (VFA, etc.) in the feed to the activated sludge reactor, and the onset of methane fermentation depleting the VFA pool must therefore be kept under control.

Shipin & Meiring (1997) observed that the PETRO fermentation pit featured high rate RBCOD production even at relatively long sludge retention time (SRT) (>15 days) combined with short hydraulic retention time (HRT) (<15 hours). Under conditions of high rate recirculation conditions a specific organic loading can be safely increased well beyond the value recommended for ponds without recirculation (0.6 kg COD.m^{-3}.d^{-1}).

2.5.1.2 Secondary (oxidation) ponds

Secondary oxidation ponds continue the process of biological degradation of residual matter still present in that portion of the primary pond effluent, which is recirculated via the sidestream. Again, a relatively large portion of the residual organic matter can be removed in this fashion. It cost-efficiently reduces the load which otherwise would be imposed on the conventional, and normally more expensive, unit process in the secondary stage.

The designer has the opportunity to select the proportional split between (i) the sidestream returning to the primary pond via oxidation ponds and (ii) the rest that proceeds to the secondary stage. On the one hand, the split determines the flow returned via the side sidestream and therefore being treated in the oxidation ponds. On the other hand, it sets the flow to be treated in the PETRO reactor. The split ratio can be optimized to meet site specific conditions.

The recirculated sidestream flow should not be less than about 40% of the raw inflow, although due care should be taken not to upset facultative stratification in the primary pond where acid fermentation is essential. An important outcome of the recirculation is enhancement of algal growth in the ponds which increases oxygenating capacity of the recycle (Shipin & Meiring, 1997).

2.5.1.3 PETRO facility

This unit process biologically entrains suspended microalgae which are invariably generated in the ponds. This aspect of algae removal from the final effluent is quite unique to PETRO. As already mentioned it can be either an activated sludge process (ASP) or a trickling filter (TF). The activated sludge variant can be extended at little extra cost to facilitate pronounced biological phosphorus removal. We will focus on the TF aspect here.

2.5.1.3.1 Tricking filters

High performance of the PETRO system relies on the effective removal of microalgae in the trickling filter, which depends on the establishment of a heterotrophic biofilm on the filter medium (Meiring et al., 1994; Meiring and Oellermann, 1995; Shipin et al., 1998).

A principal difference between the PETRO and conventional trickling filters is that a substantial portion of the organic load received by the PETRO filter is in the form of live algal biomass, which has important consequences for the TF operation. The biofilm mass in a conventional TF increases substantially in winter, due to a lower level of biological oxidation by bacteria and fungi at the lower temperatures. In contrast, the biofilm mass in the PETRO TF decreases down to 2.4 times in winter compared to its summer values (Shipin et al., 1998). This winter decrease correlates with a two-fold drop of algal concentration in the TF, suggesting that other mechanisms may be controlling biofilm production. The biofilm of a conventional TF is dominated by bacteria and/or fungi which are the major producers of the exopolysaccharide slime (Mack et al., 1975; Bruce and Hawkes, 1983); this imparts viscosity to the biofilm, enhancing immobilization of microbial consortium and preventing its wash-off. The role of microalgae in this case is thought to be limited to the marginal development on the surface exposed to the light (Wolowski, 1989).

Large numbers of microalgal species have been shown to function heterotrophically in the dark. Microalgae are thought to heterotrophically utilize low molecular weight organics such as amino acids, monosaccharides, VFA, etc. (Neilson and Lewin, 1974; Abeliovich and Weisman, 1978; Pearson et al., 1987). As a response to various stress conditions many microalgae were reported to enter the stationary growth phase and produce large quantities of EPS, a secondary metabolite, under both light and dark conditions (Ramus, 1980). It is suggested that microalgae from the secondary stabilization ponds grown mixotrophically undergo a transfer while entering the trickling filter. The transfer introduces stress conditions and cells may enter stationary growth phase which provides a stimulus for EPS overproduction (Shipin et al., 1999a). These biopolymers aggregate suspended solids and residual algal biomass and slough off the TF rock medium. The solids are removed from the system in the form of readily gravitating flocs in the downstream clarifier.

In an experiment conducted by Shipin et al., (1999a), the incorporation of radiolabelled substrates into algal biomass in the dark indicated that algae actively function heterotrophically as a part of the TF biofilm consortium. The role of microalgae in the TF was therefore not limited to the surface exposed to the light as in the case of a conventional TF, and heterotrophic algae along with bacteria appeared to be major producers of EPS in the TF. Furthermore, their results confirmed field observations that supplementation with dissolved organics is a crucial requirement for EPS production, and therefore for algae removal. Lack of dissolved organics in the algae-rich TF inflow rapidly lead to the loss of a healthy biofilm consortium.

High performance of the PETRO trickling filter appears to be based on the following natural phenomena: (i) an enhanced activity of micropredators (protozoa, rotifers) feeding on microalgae, (ii) heterotrophic activity of microalgae and bacteria feeding on dissolved organics while fungi play a minor role and (iii) conversion of dissolved organics into colloidal exopolysaccharides (Shipin et al., 1999a).

2.5.2 Application of PETRO process principles to combined algal-duckweed systems

2.5.2.1 Rock filters

Rock filters were suggested as a method for the removal of algal cells from the final effluent of the proposed combined duckweed-algal system in order to improve compliance with respect to COD and suspended solids in the effluent, as well as for the removal of any vestigial ammonia by aeration of the filters where necessary.

In 2005, Johnson & Mara investigated the use of both unaerated and aerated rock filters for the removal of both algal cells and ammonia from the final effluent through the mechanism of nitrification. The aerated filter removed >90% of both SS and BOD; its effluent BOD was consistently <5 mg/l. SS and BOD removals in the control filter were much more variable (70-90% and 45-90%, respectively); nevertheless the control filter achieved the EA requirement for both these parameters. Effluent TKN from the aerated filter was consistently <5 mg/l, whereas the unaerated control filter frequently failed to reduce TKN. A similar pattern established for ammonium removal. The NH_4^+-N concentration in the influent to both filters was reasonably similar and varied from 2 to 7 mg/l. The aerated filter effluent consistently removed NH_4^+-N to <2 mg/l, but the control filter did not remove any NH_4^+-N, in fact, its concentration generally increased. Nitrate was produced in the aerated filter, but not in the control. Nitrite was below detection levels in both the influent and effluent for both filters. Improved faecal coliform removal was also observed in the aerated rock filter.

Based on the experimental data gathered in this study, it is clear that the duckweed preferentially utilize ammonia as a nitrogen source. There is therefore unlikely to be a high concentration of ammonia in the effluent of the duckweed ponds as it enters the algal ponds. Because of the lag in the utilization of nitrate, depending on the retention time in the duckweed ponds, it is likely that the removal of any nitrate from the system will take place in the algal ponds through algal uptake. Any ammonia remaining in the duckweed pond effluent will likely be nitrified by heterotrophic bacteria under the aerobic conditions of the algal ponds. It is therefore unlikely that the use of aerated rock filters for the nitrification of ammonia in the final effluent will be necessary.

In terms of the removal of algal cells from the final effluent, it can be seen from the information on trickling filters of the PETRO process that algae that are functioning heterotrophically in the dark play an important role through the production of EPS. The EPS is responsible for the further entrapment of the algal cells in the algal pond effluent into the biofilm matrix. A good supply of readily biodegradable organics such as VFAs and amino acids are necessary in order for the algae and bacteria comprising the biofilm to produce sufficient EPS for this to take place. This supply of organics is guaranteed in the PETRO process by supplying a portion of the primary pond effluent directly to the trickling filter. In a combined duckweed-algal system, it is unlikely that there will be a sufficient supply of these readily available organics in the final effluent to ensure a healthy EPS producing biofilm. COD removal is expected to be efficient in the system through anaerobic breakdown in the primary pond, followed by consumption of COD by sulphate reducing bacteria in the duckweed ponds, and finally uptake of the remaining readily biodegradable organics in the algal ponds.

2.5.3 Alternative algal cell removal strategy

As an alternative to the rock filters that were initially proposed for the removal of algal cells from the final effluent, it is suggested that a final duckweed pond be implemented after the algal ponds and before discharge of the final effluent (Figure 2-81). Results of the current study indicate that duckweed are capable of survival at very low nutrient concentrations (less than 0.2 mg/l, 0.3 mg/l and 0.4 mg/l NH_4-N, NO_3-N and PO_4-P respectively), and even continue to take up nutrients at these low concentrations. Under these conditions, the surface area of the plants increased and the growth rate decreased significantly, but the net surface area covered by the plants under long culture ages was the same as those at higher nutrient concentrations. It is therefore proposed that the final pond not be harvested, or only be harvested occasionally. This will ensure a thick mat of duckweed to exclude light from the water. The shading effect of the duckweed will result in the death or senescence and sedimentation of the algal cells, resulting in a clear effluent. Although the nutrient concentrations will be low by this point, the duckweed will serve a secondary purpose of removing any remaining ammonia.

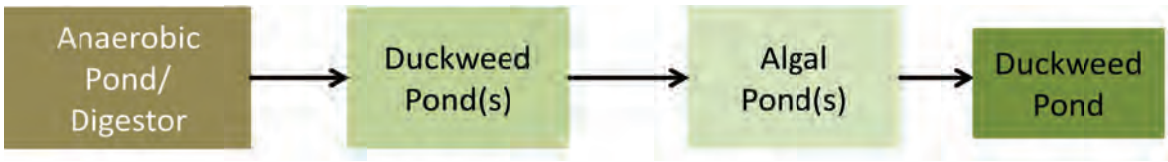

Figure 2-81: Suggested sequence of unit processes for optimum nutrient removal and effluent polishing

Because of the anaerobic conditions that are expected to develop in the final duckweed pond even under low conditions of COD, as was experienced in the laboratory studies, it may be necessary to aerate the final effluent, either mechanically, or by natural re-aeration over a rockery or waterfall before discharge.

Because of the difficulty in determining the algal re-growth potential after the duckweed ponds under laboratory conditions, it is proposed that the concept of a final duckweed pond as an algal removal mechanism be tested during a full scale trial.

2.6 Application to full scale systems

Based on the research that has been conducted to date, and the assessments of existing duckweed systems in the Limpopo Province, the following design considerations have been noted; These will be used to prepare a conceptual design for a full scale combined algal-duckweed system, including anaerobic digestion.

- The expected effluent of anaerobic digester must be considered, in order to determine the risk of toxicity to the duckweed population under high nutrient concentrations.
- The main function of duckweed is the removal of nitrogen from the system; the full scale design should aim to remove certain mass of nitrogen based on biomass composition.
- The mat density is important for the attenuation of light and the prevention of algal growth. The goal should be to try to operate plant in exponential growth phase so that ratio of dead plants to living plants is low. The light intensity, nutrient concentration and temperature should be considered so as to prevent the duckweed population from entering a resting state, for example the formation of turions in turion-forming species. This may limit the application of this system to certain climatic conditions and geographical areas.
- Based on the observations made in the reactor experiments, it is clear that a full scale plant should be designed with baffles. The number of baffles to the reactor volume is important so as to get the optimum nutrient exposure to the duckweed layer, and to prevent diffusion limiting conditions. The reactors used for the study had a high recirculation rate, and assisted with dispersion without creating surface turbulence. Diffusion of oxygen through surface in reactors was minimal despite a high recirculation rate.

- The nutrient removal from the system is determined by the duckweed surface area, however the residence time is the most important factor for COD removal. The full scale system should therefore be designed based on the surface area to volume ratio.
- Serpentine or orbal reactors could be considered for the ease of mixing and harvesting. This will allow for random harvesting to prevent the removal of the same section of the duckweed mat, ensuring a uniform culture age. The population would also circulate, preventing one portion of the population from being exposed to continued high nutrient concentrations that may be limiting.
- Overflow structures are important to allow for either retention or wash-out of duckweed.
- A plug flow system with step feed and recirculation could be considered; step feed will prevent a localized concentration gradient in the system, and the recirculation will reduce toxicity of high nutrient concentrations by diluting inflow.
- Results show that duckweed can assimilate nutrients at very low concentrations, so a slow growth rate is not necessarily indicative of poor nutrient uptake. A final duckweed "effluent polishing" pond could be considered after the algal ponds. This will also remove suspended solids. The final effluent may require physical aeration, but rock filters will no longer be necessary for algal biomass removal.
- Harvesting and handling of wet biomass should be considered. Various options for duckweed harvesting have been described in literature; these will be reviewed and considered for application in the South African context.

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CHAPTER 3 CONCEPTUAL DESIGN AND FURTHER WORK

3.1 Introduction

Conventional wastewater treatment systems are expensive in either investment or running costs. On the other hand, waste stabilisation ponds may be unable to meet effluent standards for nutrients. Wastewater treatment using duckweed together with algae presents a wastewater treatment option capable of achieving effluent standards and generating revenue from selling the duckweed. However, despite the potential for combined duckweed-algal treatment systems to meet the required standards for organic material, nutrients and pathogens, simply adapting current waste stabilization pond designs to accommodate duckweed treatment stages may have a high land requirement, and therefore may not be a cost effective option. Improved guidelines for the design of duckweed-based systems are therefore necessary to obtain a reliable and cost-effective wastewater treatment plant using duckweed. An optimally designed combination of anaerobic ponds, duckweed systems and algal maturation ponds can minimise land requirements and capital costs while achieving specified effluent standards.

As discussed in section 2.6 of Chapter 2, the results of the laboratory scale study highlighted some important considerations for the design of a full scale system. Conceptual designs will be presented here, each of which will need to be tested on a pilot scale before the final full scale design can be finalized. A summary of the important design considerations are as follows:

- The high concentration of nutrients in the anaerobic digester effluent may be toxic to the duckweed. A plug flow system with step feed and recirculation could be considered; step feed will prevent a localized concentration gradient in the system, and the recirculation will reduce toxicity of high nutrient concentrations by diluting inflow.
- It is important that the duckweed layer not be diffusion limited, but nor should the surface layer be disturbed by turbulence. This can be achieved with baffles.
- The main function of duckweed is the removal of nitrogen from the system; the full scale design should aim to remove certain mass of nitrogen based on biomass composition. P will be proportionally removed.
- The correct mat density is important for the attenuation of light and the prevention of algal growth, but the density should not be allowed to increase to a point where the plants become light, nutrient and gas transfer limited.

- The light intensity, nutrient concentration and temperature should be considered so as to prevent the duckweed population from entering a resting state, for example the formation of turions in turion-forming species. This may limit the application of this system to certain climatic conditions and geographical areas.
- The nutrient removal from the system is determined by the duckweed surface area; however the residence time is the most important factor for COD removal. The full scale system should therefore be designed based on the surface area to volume ratio.
- Culture age is important; new fronds should not be harvested until they have had the opportunity to multiply. Serpentine or orbal reactors could be considered for the ease of mixing and harvesting. This will allow for random harvesting to prevent the removal of the same section of the duckweed mat, ensuring a uniform culture age. The population would also circulate, preventing one portion of the population from being exposed to continued high nutrient concentrations that may be limiting.
- Overflow structures are important to allow for either retention or wash-out of duckweed.
- Results show that duckweed can assimilate nutrients at very low concentrations. A final duckweed "effluent polishing" pond could be considered after the algal ponds. This will also remove suspended solids. Aeration of the anaerobic effluent may be required, for example using a step cascade, since anaerobic conditions are expected to develop underneath the floating cover.
- Harvesting and handling of wet biomass should be considered.

Important design considerations are also available in literature, especially regarding expected yield and recommended retention times. Alaerts et al. (1996) conducted detailed sampling of a duckweed lagoon system during the dry season, when the hydraulic retention time was 20.4d. At two-thirds of retention time the plants had absorbed virtually all NH_4^+ and ortho-PO_4^{3-} from the water column. The authors estimated that the duckweed harvest would remove 60-80% of the N and P load in a water tight lagoon, or 0.26 g N/m^2.d and 0.05 g P/m^2.d in the first three-quarters of retention time.

Al-Nozaily et al. (2000) assessed the effect of depth, mixing and nutrient concentration on duckweed growth. The authors found that at a low nitrogen surface loading concentration of 183 kg N/ha, TN removal (uptake plus losses) could be completely attributed to duckweed uptake. TN and TP removal rates attributed to duckweed ranged from 1-4.8 kg N/ha.d^{-1} and 0.13-0.58 kg P/ha.d^{-1}.

In a pilot study conducted by El-Shafai et al. (2007), an up-flow anaerobic sludge blanket (UASB) reactor was followed by three duckweed ponds containing *Lemna gibba*. During the

warm season, removal efficiencies of ammonia, TKN and total phosphorus were 98%, 85% and 78%, with residual concentrations of 0.41 mg N/l, 4.4 mg N/l and 1.11 mg P/l respectively. 80% of N removal was by plant uptake and conversion to protein, on average 4.42 kgN/ha.d. The total P recovered by duckweed ranged from 0.97 kg P/ha.d in the first duckweed pond, 0.94 kg P/ha.d in the second pond and 0.86 kgN/ha.d in the third pond. In winter, the TN recovered by the plants was 1.21, 1.46 and 1.28 kg N/ha.d in the three duckweed ponds respectively, and the TP recovery values were 0.27, 0.32 and 0.29 kg P/ha.d respectively.

3.2 Conceptual designs

In order to address the issues above, the following conceptual designs are proposed. All designs include an anaerobic digestor or pond, a duckweed treatment phase, a series of algal maturation ponds, a duckweed polishing pond and final effluent aeration and discharge. The designs are differentiated by the design of the duckweed treatment stage and the presence or absence or step feed and/or recirculation.

Each of the proposed systems will need to be tested under pilot scale conditions in order to determine which system will give the optimal balance between efficiency and cost effectiveness.

3.2.1 Baffled duckweed ponds with single influent point

The first proposed conceptual design includes a simple duckweed treatment stage, with a single influent point and baffles (Figure 3-1). Baffles will ensure that the duckweed layer is not diffusion limited, by generating turbulence in the system. In this system there is no way to control the concentration of nutrients supplied to the duckweed from the anaerobic digestor.

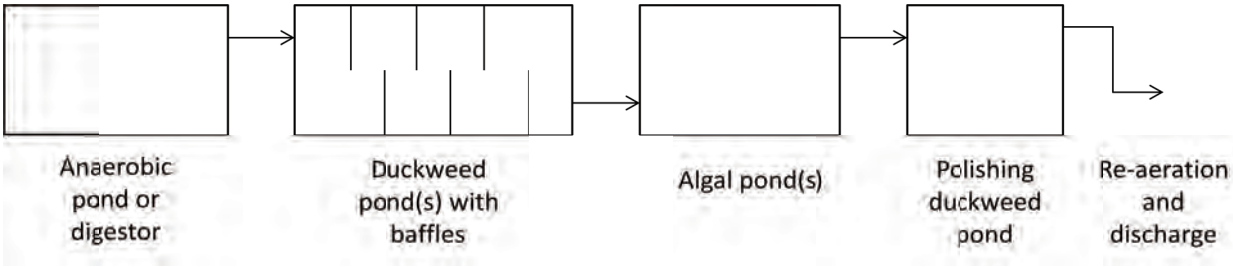

Figure 3-1: Baffled duckweed pond with single influent point

3.2.2 Baffled duckweed ponds with step feed

In order to prevent a situation where the duckweed in the initial section of the treatment phase are exposed to a higher localised nutrient concentration than the rest of the pond, a step-feed system can be introduced into the duckweed treatment pond (Figure 3-2).

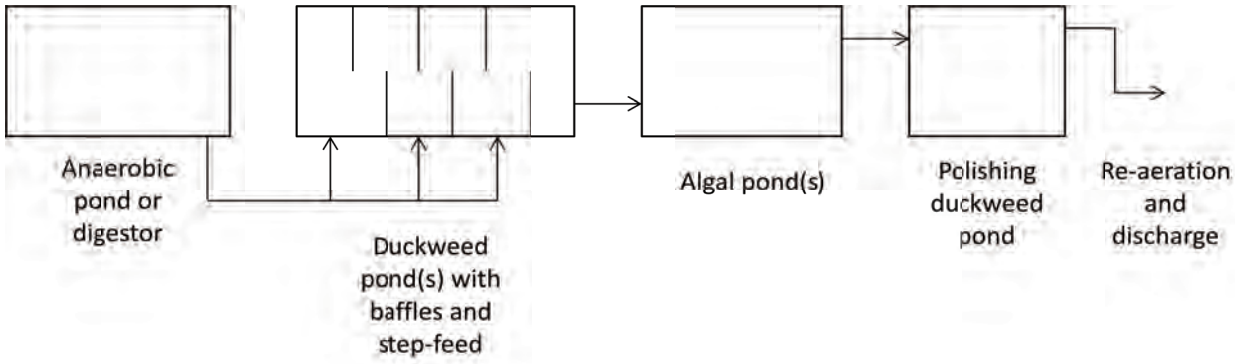

Figure 3-2: Baffled duckweed pond with step feed

3.2.3 Baffled duckweed ponds with step feed and recirculation (duckweed pond effluent only)

In order to afford the operator an element of control over the concentration of nutrients in the feed, recirculation of the duckweed pond effluent can be introduced, thereby reducing the risk of toxicity of high nutrient concentrations (Figure 3-3).

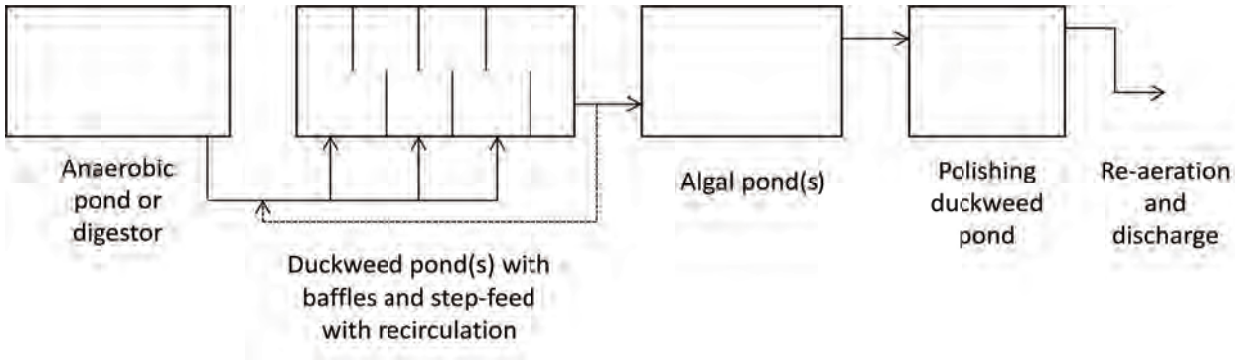

Figure 3-3: Baffled duckweed pond with step-feed and recirculation of duckweed pond effluent

3.2.4 Baffled duckweed ponds with step feed and recirculation (combined duckweed pond effluent and final effluent)

Further dilution of the nutrient concentration in the digestor effluent can be achieved by introducing a recirculation line from the final effluent (Figure 3-4).

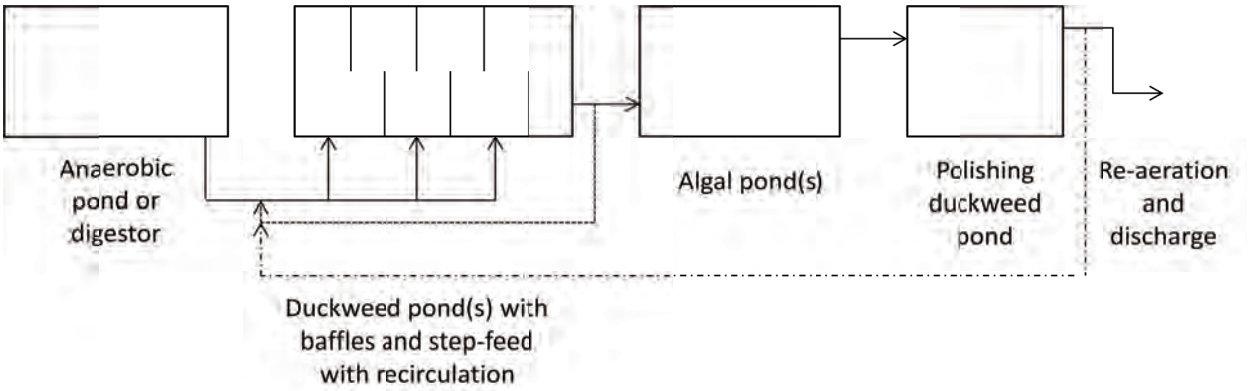

Figure 3-4: Baffled duckweed pond with step-feed and recirculation of duckweed pond effluent and final effluent

3.2.5 Orbal duckweed pond with mechanical mixing and recirculation

In a situation where a mechanical option can be considered, an orbal duckweed reactor with mechanical mixers for constant recirculation may provide a means to save land space, as well as introduce automated harvesting (Figure 3-5). Gentle mixing, perhaps below the surface so as not to disturb the surface layer, could be applied to ensure continuous movement of the duckweed layer around the reactor. Step feed would not be required in this case, as the circulating plants would be exposed in turn to high and low concentrations of nutrients, preventing acclimatisation.

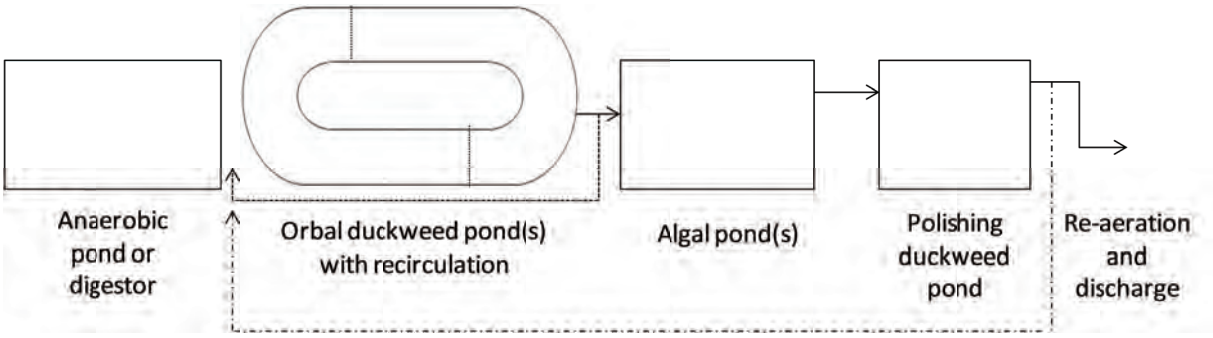

Figure 3-5: Orbal duckweed pond with mechanical mixers, and recirculation of duckweed pond effluent and final effluent

3.2.6 Overflow weir design

The design of the overflow weirs of the duckweed and algal ponds will play an important role in determining whether algae or duckweed become dominant. A suggested overflow weir design is presented in Figure 3-6. If abstraction of water is from the surface from one pond to the next, this will encourage the overflow and washout of duckweed, allowing algae to become dominant. However, if the weir is designed so that abstraction of water is from

below the surface, the duckweed surface layer can remain undisturbed and therefore will become dominant in the pond, excluding the algae through the attenuation of light.

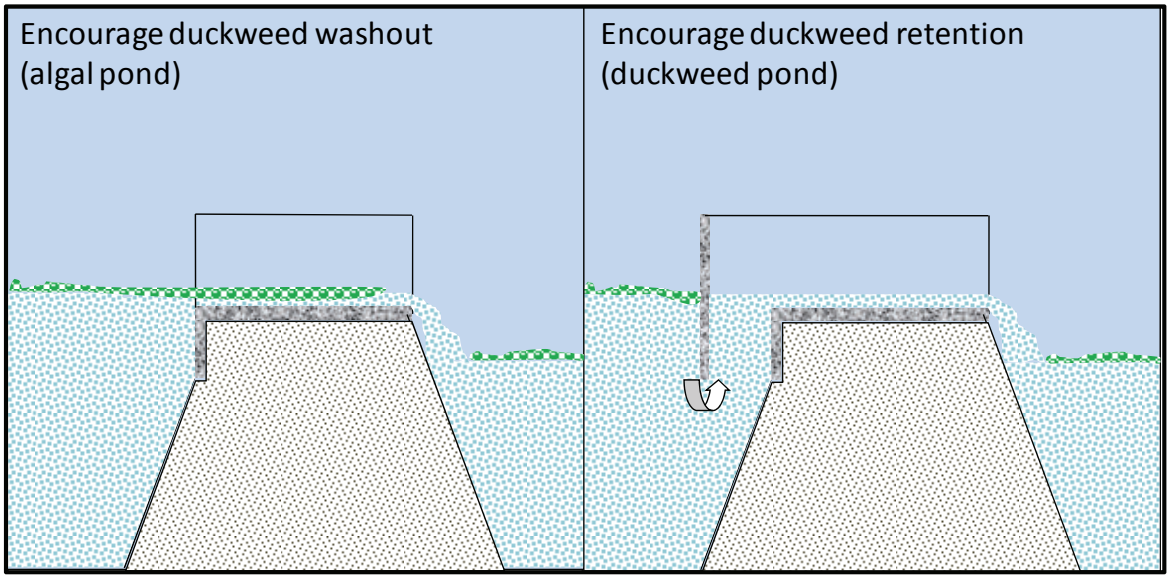

Figure 3-6: Suggested weir design for the wash out (left) or retention (right) of duckweed, giving rise to algal ponds of duckweed ponds respectively

3.3 Further research requirements

Although the laboratory studies that have been conducted in this phase of the project have provided valuable insight into the consideration for the design of combined duckweed-algae treatment systems, it is clear that further research is required on a pilot scale in order to test the conceptual designs describe above. Pilot trials should focus on the following aspects in order to design a full scale system with confidence;

- The algal re-growth potential in the algal ponds following the duckweed system must be determined. It was not possible to test this in the laboratory scale trials.
- The factors and specific conditions that affect the formation of turions or resting bodies by the duckweed must be determined with more confidence, as turion formation is to be avoided at all costs if the system is to be successful. This will likely play a role in the selection of suitable sites for the application of the technology.
- The conceptual designs that have been proposed must be tested under different conditions in order to determine which is the most effective configuration for specific applications
- Harvesting and drying of the duckweed plants on a large scale is an important consideration, and methods for this must be tested and developed in a pilot scale phase.
- A thorough investigation into the duckweed species found naturally in various regions of South Africa should be undertaken, and the different species should be

tested in order to determine which species would have higher growth and nutrient uptake rates under different climatic conditions.

- In addition to the design criteria, a pilot scale phase will give more insight into the expected operational requirements for these combined systems, and will enable the compilation of a definitive guide. A guide for the assessment of current waste stabilization pond systems to determine their suitability for conversion to a combined duckweed-algal-based system will also be developed.

3.4 References

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Appendix A: Supplementary Data

Table A-1: Duckweed cultures grown at 25°C in 1/5 Huttner media

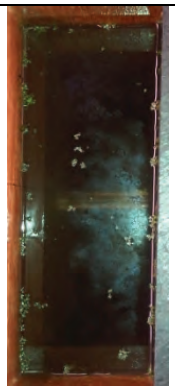
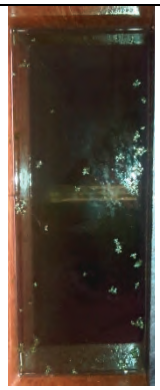
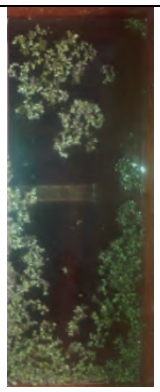
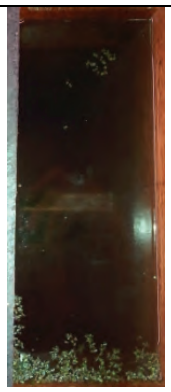
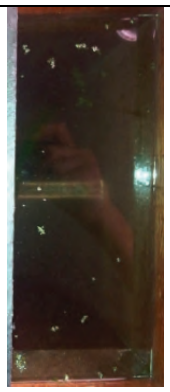
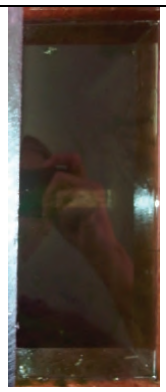
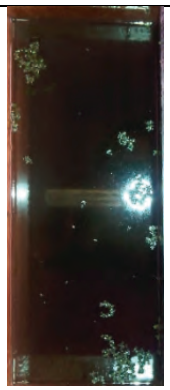
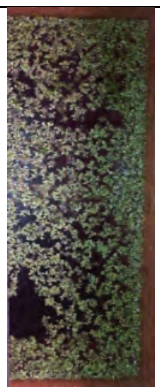
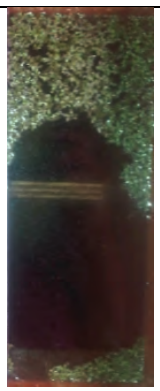
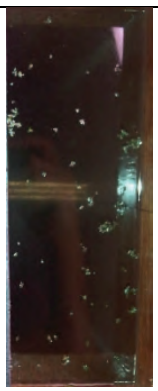
Culture Age	21 days	26 days	33 days	35 days	40 days
12d					
23d					
35d					
47d					

Table A-2: Duckweed cultures grown at 25°C in 1/25 Huttner media

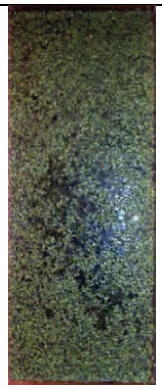
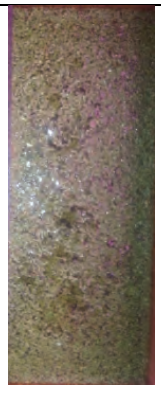
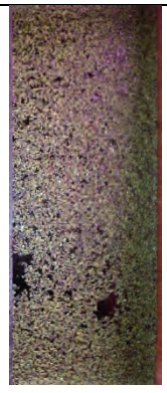
Culture Age	21 days	26 days	33 days	35 days	40 days
12d					
23d					
35d					
47d					

Table A-3: Duckweed cultures grown at 25°C in 1/100 Huttner media

Culture Age	21 days	26 days	33 days	35 days	40 days
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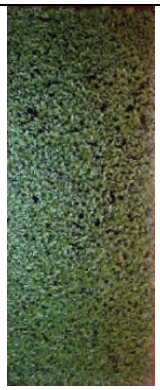
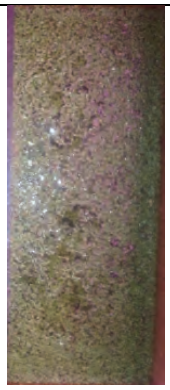
23d					
35d					
47d					

Table A-4: Duckweed cultures grown at 25°C in 1/150 Huttner media

Culture Age	15 days	21 days	23 days	28 days	32 days
12d					
23d					
35d					
47d					

Table A-5: Duckweed cultures grown at 25°C in 1/200 Huttner media

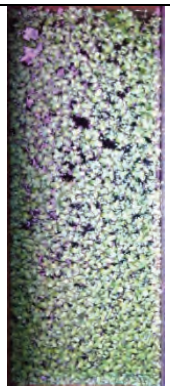
Culture Age	15 days	21 days	23 days	28 days	32 days
12d					
23d					
35d					
47d					

Table A-6: Duckweed cultures grown at 18°C in 1/5 Huttner media

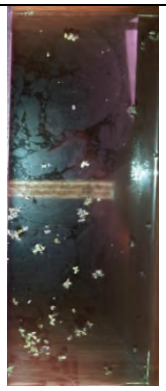
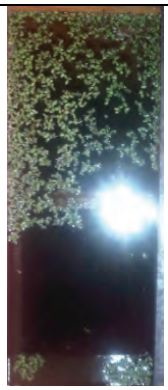
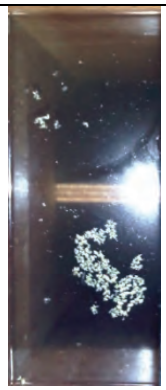
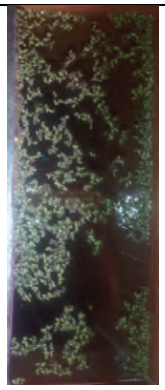
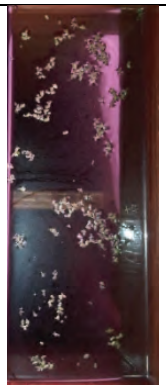
Culture Age	21 days	26 days	33 days	35 days	40 days
23d					
35d					
47d					
58d					

Table A-7: Duckweed cultures grown at 18°C in 1/25 Huttner media

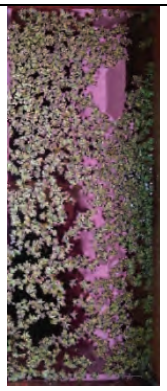
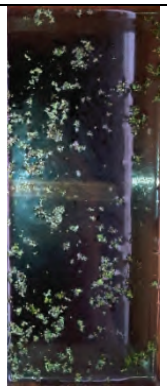
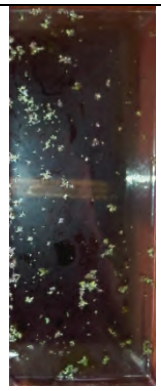
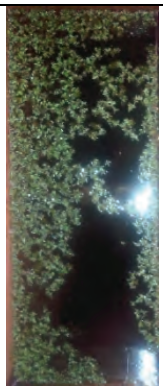
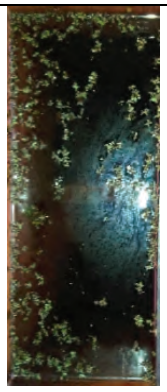
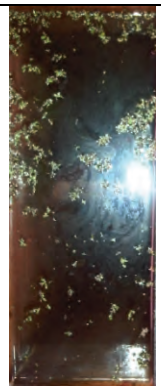
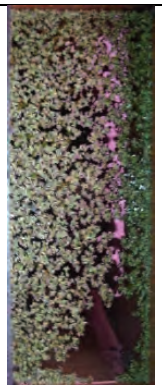
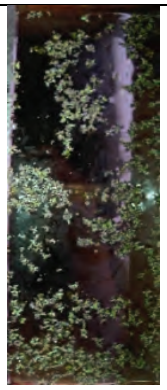
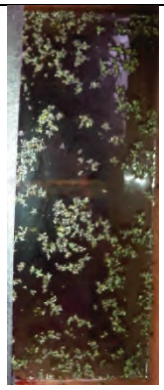
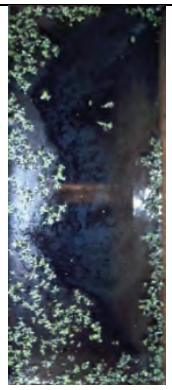
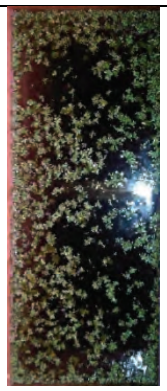
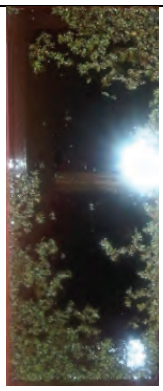
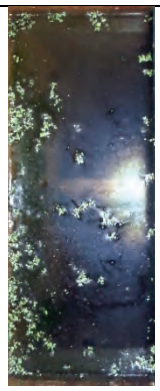
Culture Age	21 days	26 days	33 days	35 days	40 days
23d					
35d					
47d					
58d					

Table A-8: Duckweed cultures grown at 18°C in 1/100 Huttner media

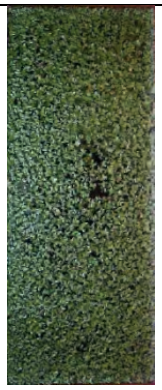
Culture Age	21 days	26 days	33 days	35 days	40 days
23d					
35d					
47d					
58d					

Table A-9: Duckweed cultures grown at 18°C in 1/150 Huttner media

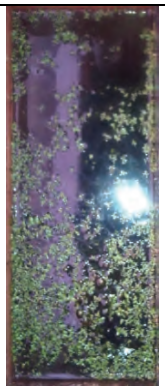
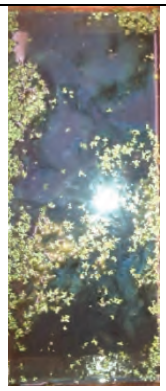
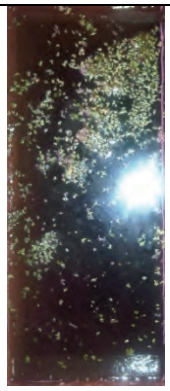
Culture Age	15 days	21 days	23 days	28 days	32 days
12d					
23d					
35d					
47d					

Table A-10: Duckweed cultures grown at 18°C in 1/200 Huttner media

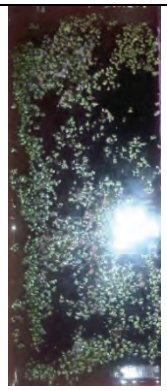
Culture Age	15 days	21 days	23 days	28 days	32 days
12d					
23d					
35d					
47d					

Table A-11: Duckweed cultures grown at 13°C in 1/5 Huttner media

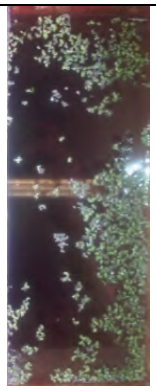
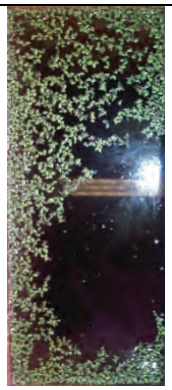
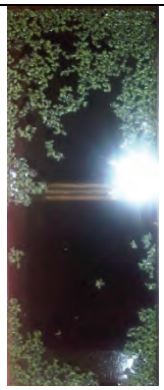
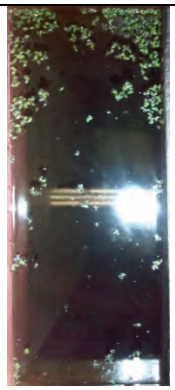
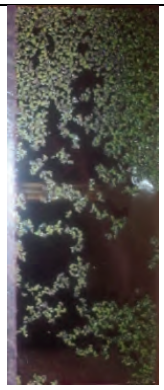
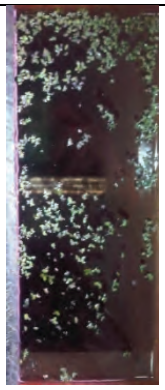
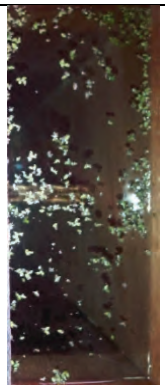
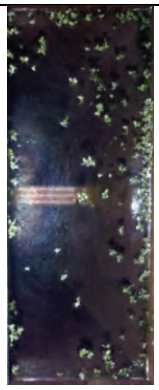
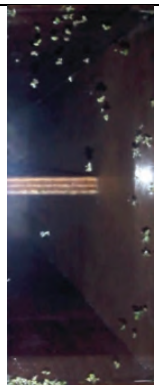
Culture Age	21 days	26 days	33 days	35 days	40 days
23d					
35d					
47d					
58d					

Table A-12: Duckweed cultures grown at 13°C in 1/25 Huttner media

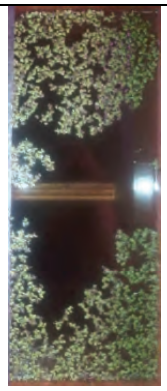
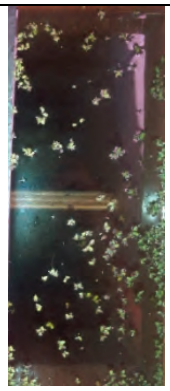
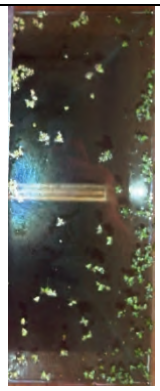
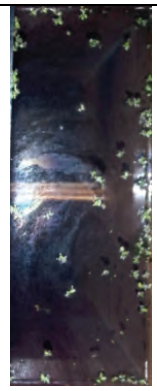
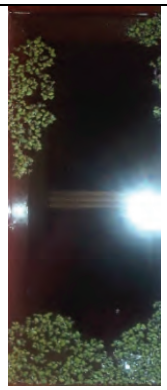
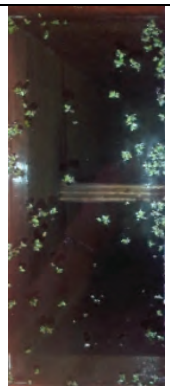
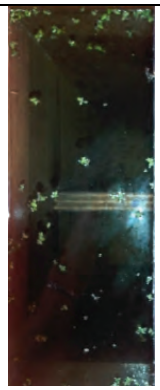
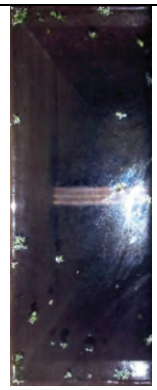
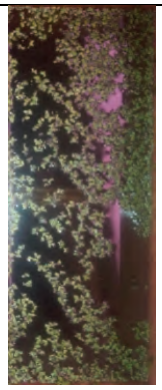
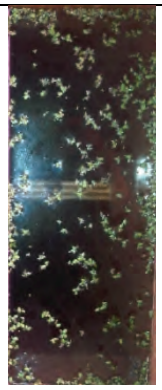
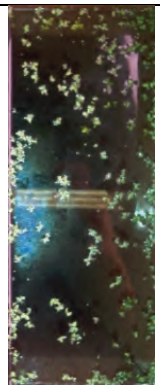
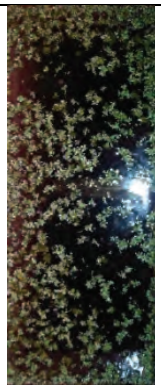
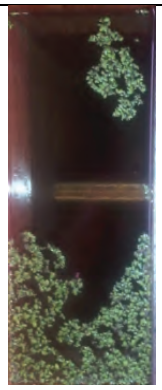
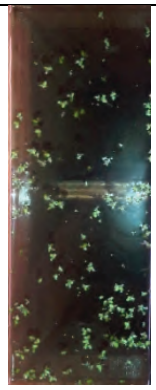
Culture Age	21 days	26 days	33 days	35 days	40 days
23d					
35d					
47d					
58d					

Table A-13: Duckweed cultures grown at 13°C in 1/100 Huttner media

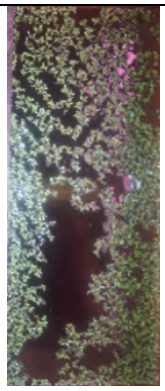
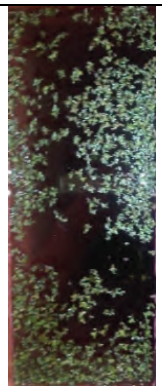
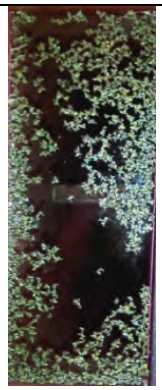
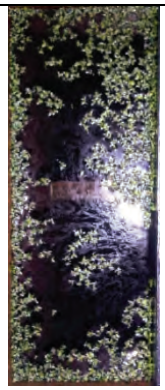
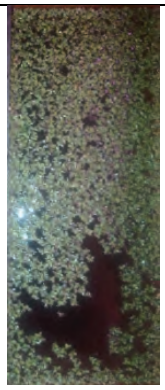
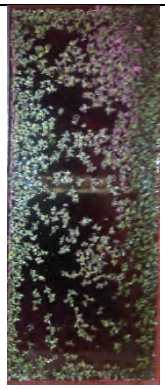
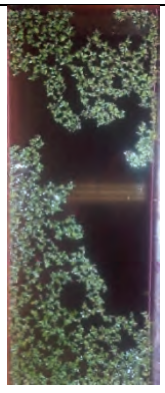
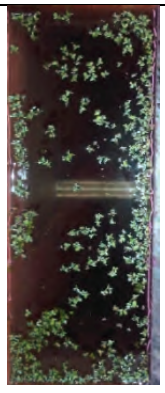
Culture Age	21 days	26 days	33 days	35 days	40 days
23d					
35d					
47d					
58d					

Table A-14: Duckweed cultures grown at 13°C in 1/150 Huttner media

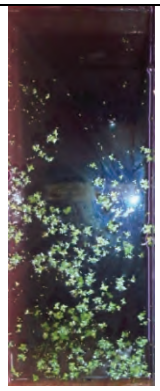
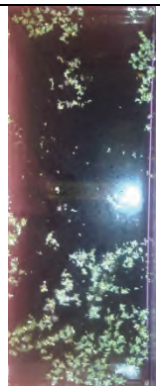
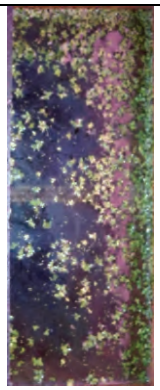
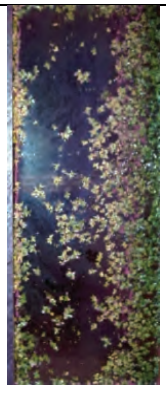
Culture Age	15 days	21 days	23 days	28 days	32 days
12d					
23d					
35d					
47d					

Table A-15: Duckweed cultures grown at 13°C in 1/200 Huttner media

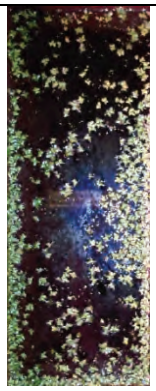
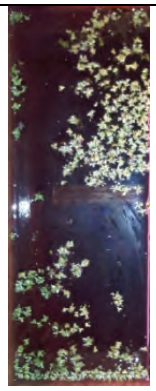
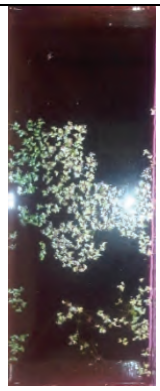
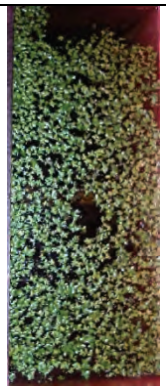
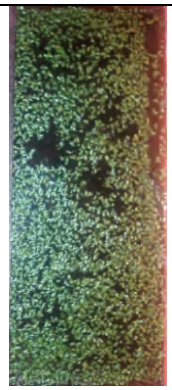
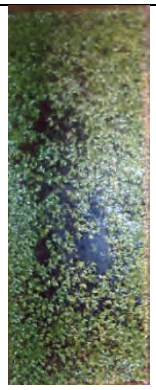
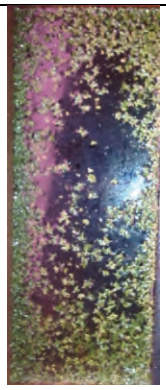
Culture Age	15 days	21 days	23 days	28 days	32 days
12d					
23d					
35d					
47d					

Table A-16: Duckweed cultures grown in the shade in 1/5 Huttner media

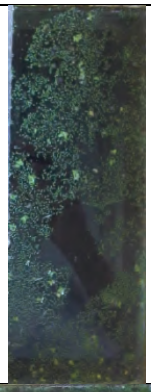
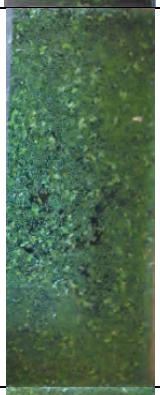
Culture Age	1d	7d	12d	19d	26d
7d					
12d					
23d					
35d					

Table A-17: Duckweed cultures grown in the shade in 1/25 Huttner media

Culture Age	1d	7d	12d	19d	26d
7d					
12d					
23d					
35d					

Table A-18: Duckweed cultures grown in the shade in 1/100 Huttner media

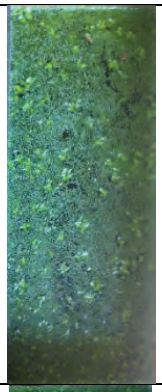
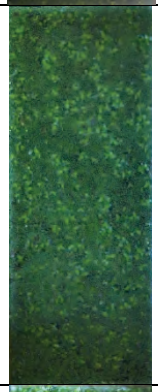
Culture Age	1d	7d	12d	19d	26d
7d					
12d					
23d					
35d					

Table A-19: Duckweed cultures grown in the sun in 1/5 Huttner media

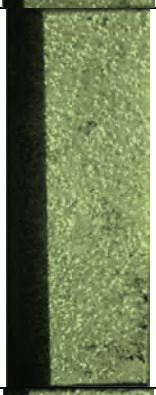
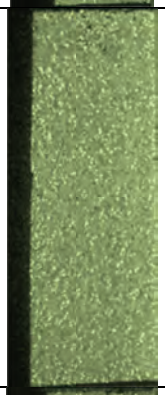
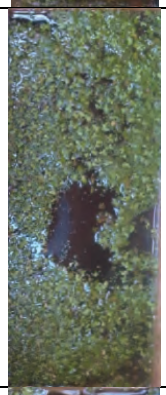
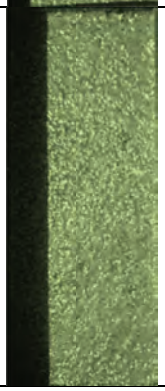
Culture Age	1d	7d	12d	19d	26d
7d					
12d					
23d					
35d					

Table A-20: Duckweed cultures grown in the sun in 1/25 Huttner media

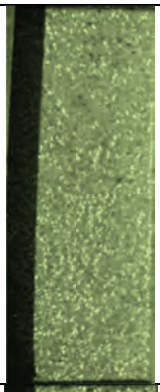
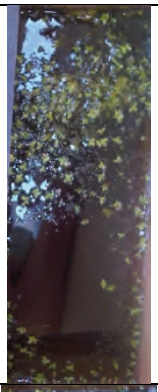
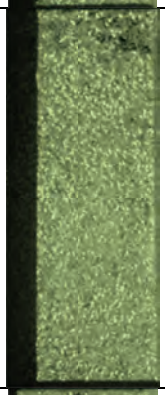
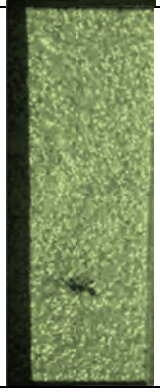
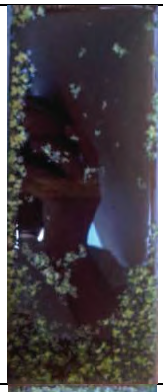
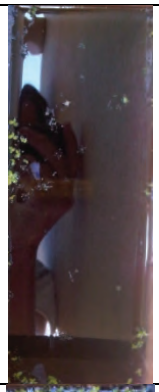
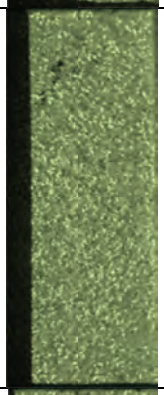
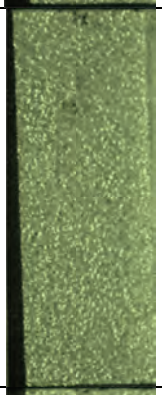
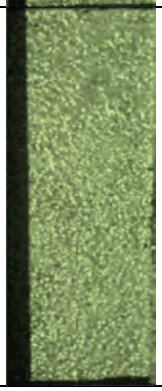
Culture Age	1d	7d	12d	19d	26d
7d					
12d					
23d					
35d					

Table A-21: Duckweed cultures grown in the sun in 1/100 Huttner media

Culture Age	1d	7d	12d	19d	26d
7d					
12d					
23d					
35d					

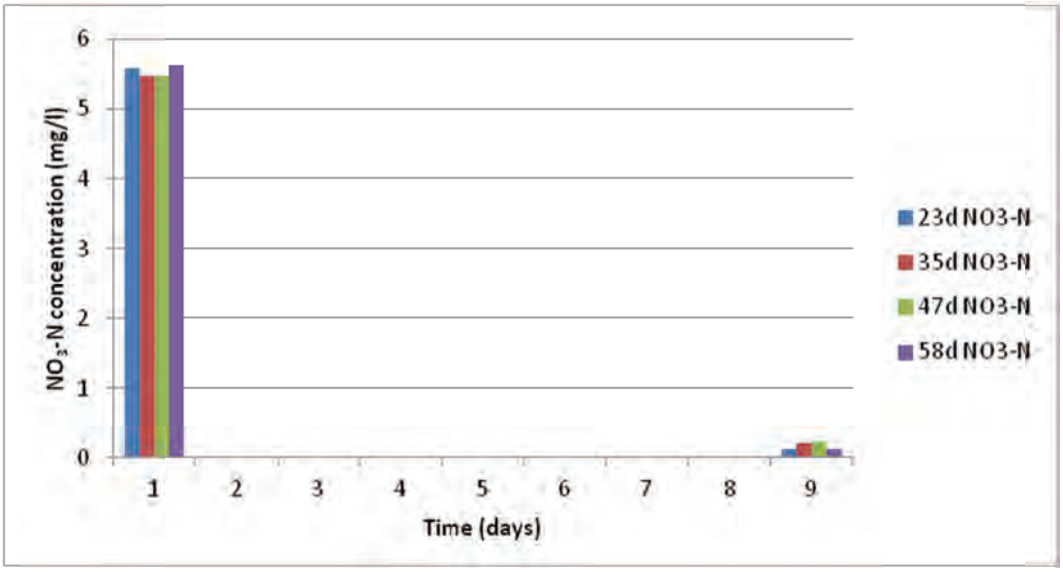


Figure A-1: Nitrate depletion from 1/25 Huttner media solution at 18°C at different harvesting rates

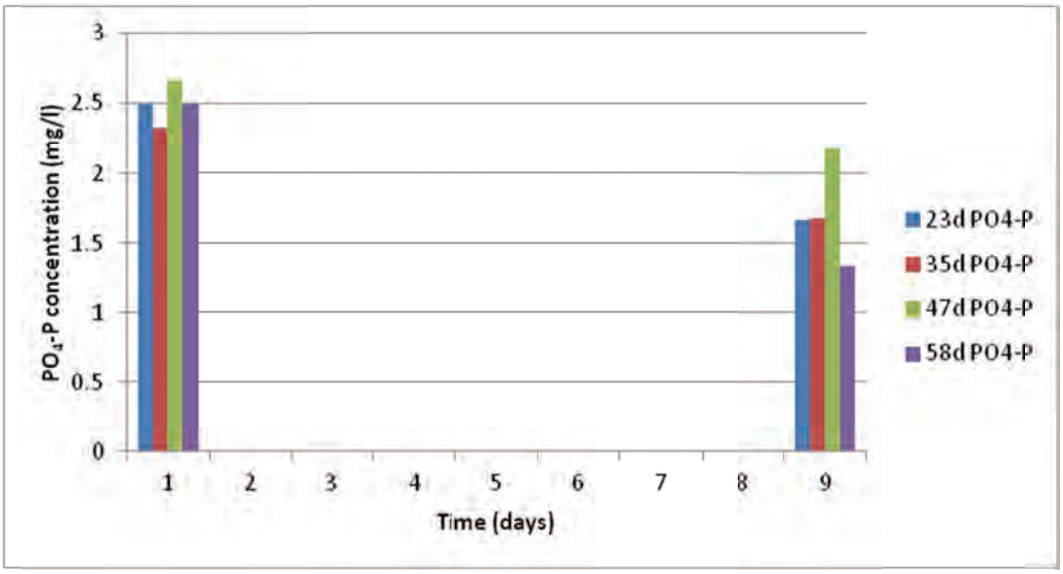


Figure A-2: Ortho-phosphate depletion from 1/25 Huttner media solution at 18°C at different harvesting rates

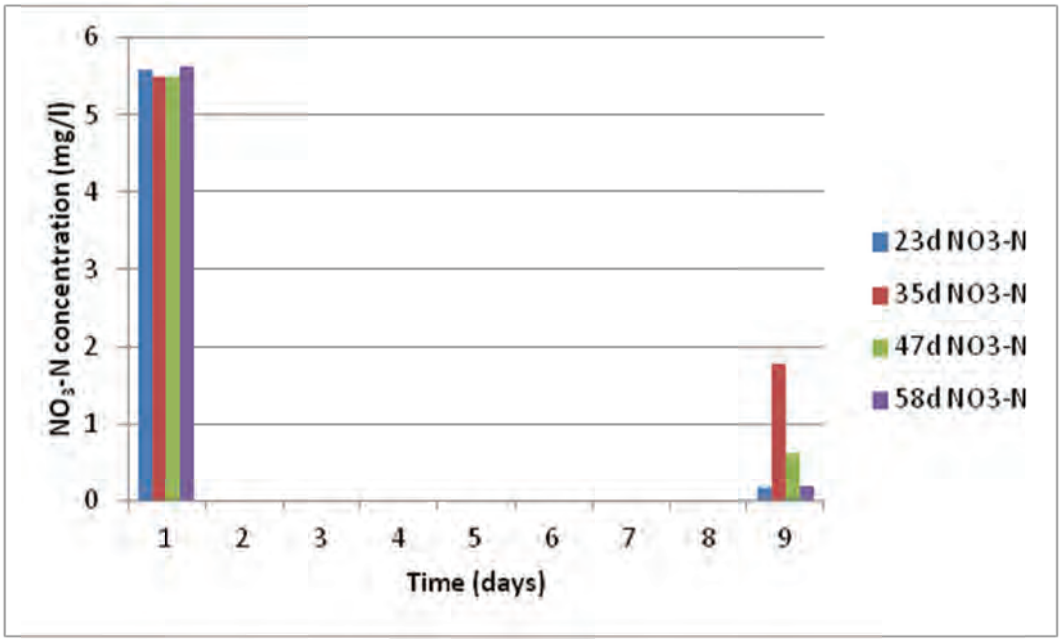


Figure A-3: Nitrate depletion from 1/25 Huttner media solution at 18°C at different harvesting rates

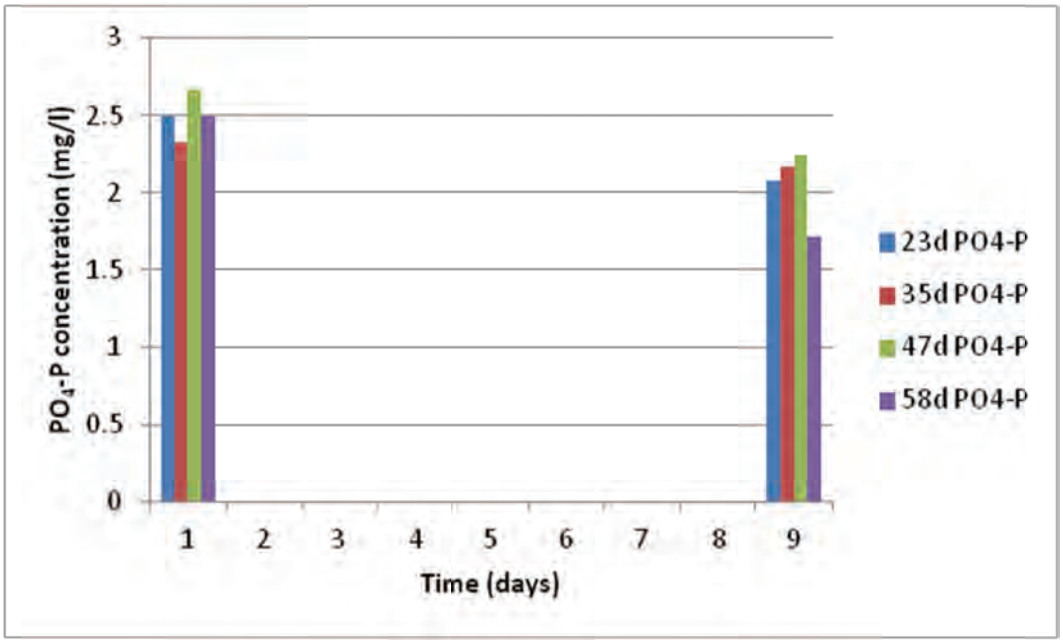


Figure A-4: Ortho-phosphate depletion from 1/25 Huttner media solution at 18°C at different harvesting rates

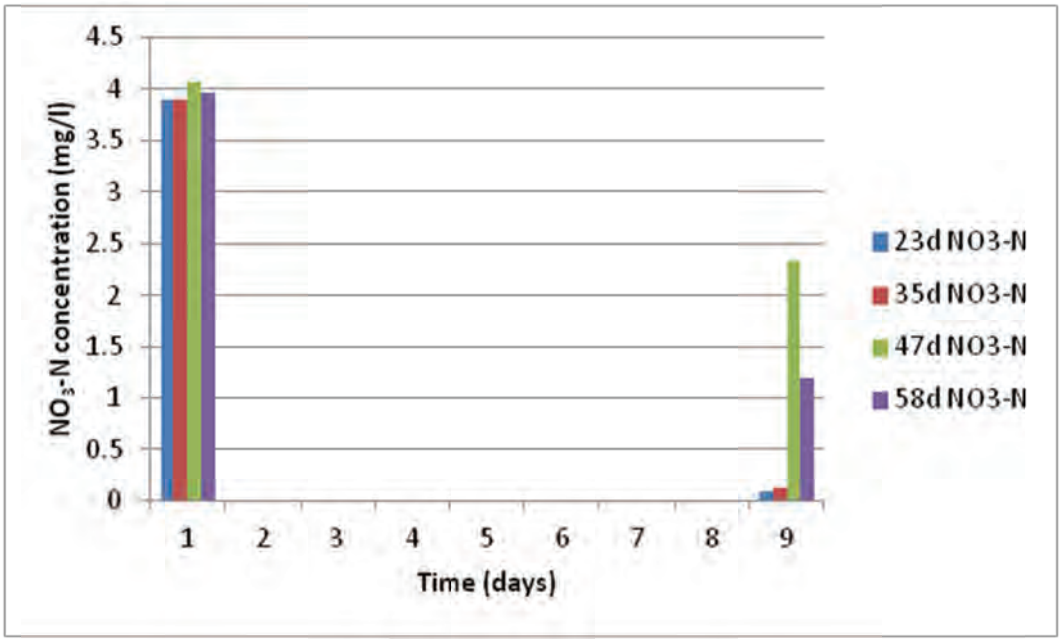


Figure A-5: Nitrate depletion from 1/100 Huttner media solution at 18°C at different harvesting rates

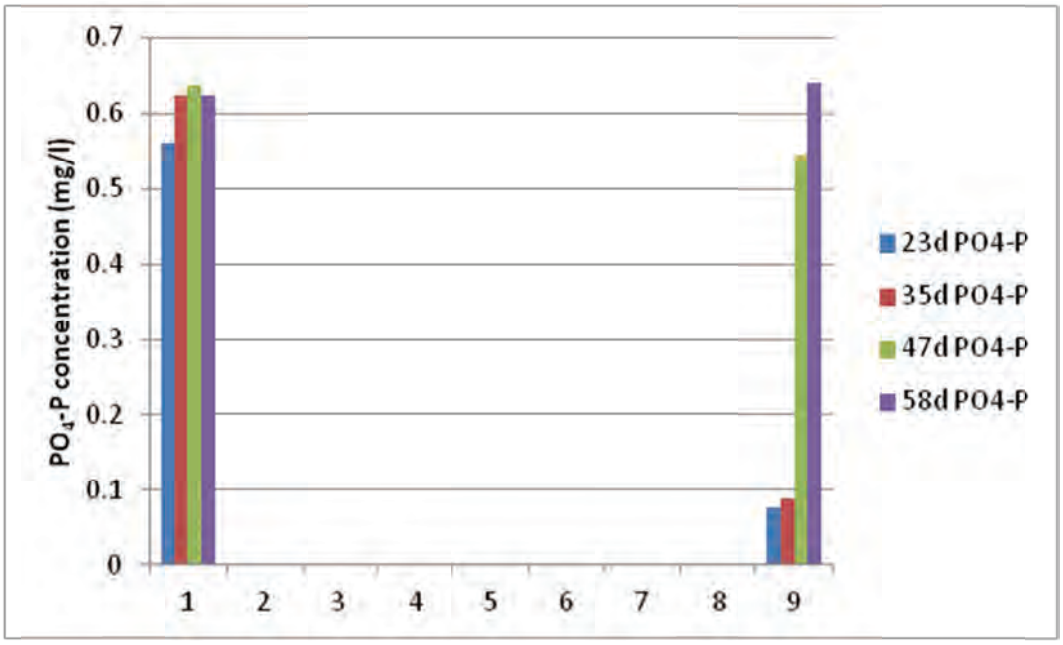


Figure A-6: Ortho-phosphate depletion from 1/100 Huttner media solution at 18°C at different harvesting rates

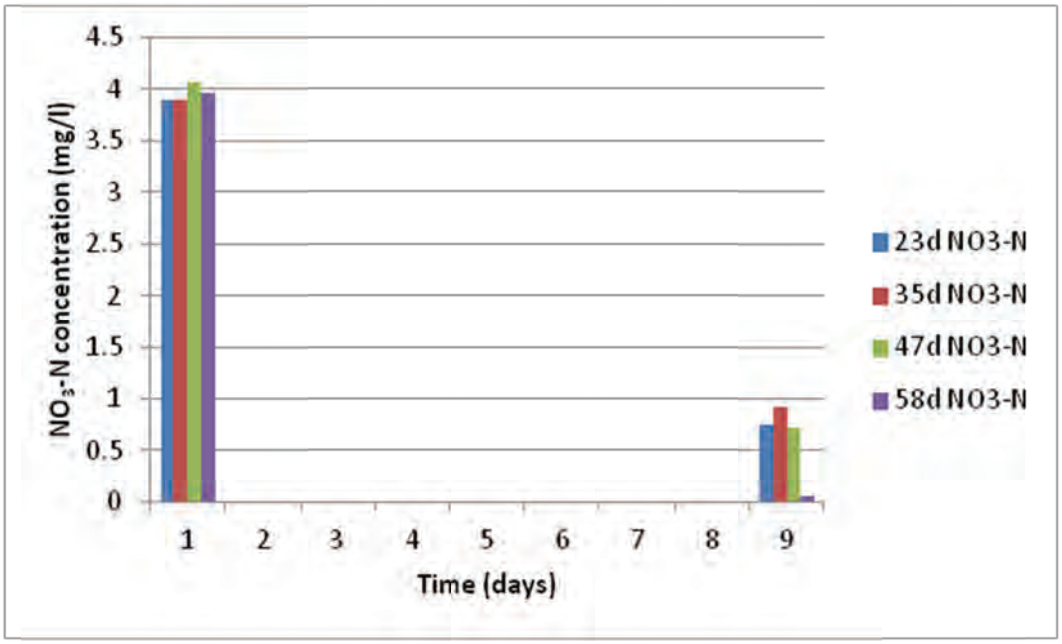


Figure A-7: Nitrate depletion from 1/100 Huttner media solution at 13°C at different harvesting rates

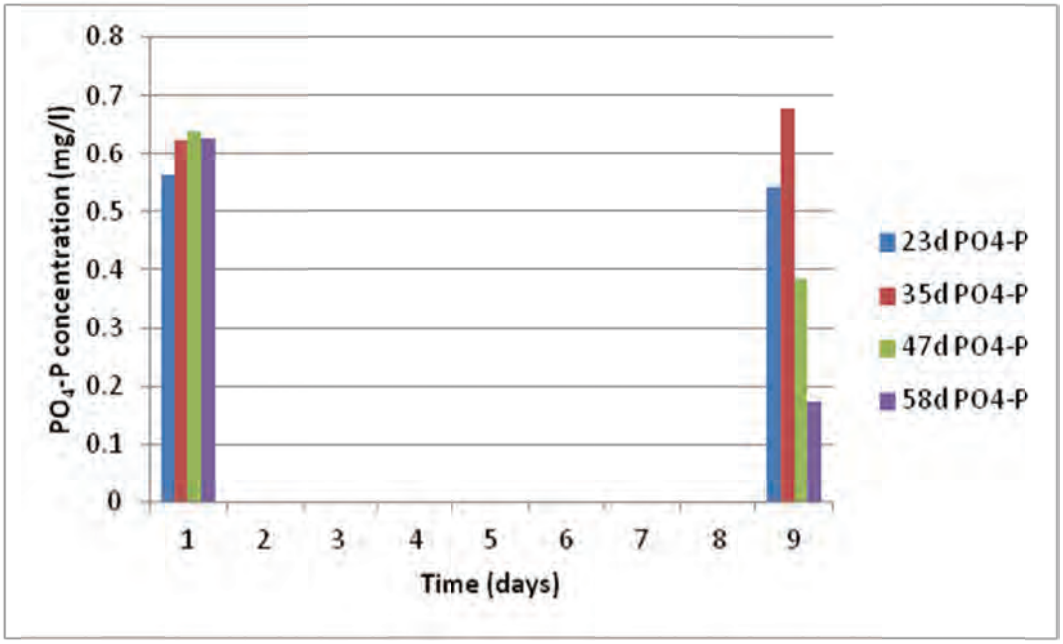


Figure A-8: Ortho-phosphate depletion from 1/100 Huttner media solution at 13°C at different harvesting rates

Appendix B: Draft Operations and Maintenance Guide

This is a draft operations and maintenance guide for combined duckweed-algal-based systems. This guide will be finalized once the pilot scale trials have been conducted in the next phase of the project.

B-1 Introduction

As with all waste water treatment facilities, the operation and maintenance of a waste stabilization pond system is crucial to ensure proper functioning, a long system life and protection of the environment and surrounding communities through discharge of compliant effluent. A well-defined manual is therefore important, as it will provide site supervisors with an understanding of the system and the operations and maintenance requirements. This in turn will empower supervisors to provide the necessary training and equipment to the on-site operations personnel.

A useful guide for the operation and maintenance of waste stabilization pond systems was developed by de Sousa and Jack (2010) for the Water Research Commission (WRC report number TT 472/10). Their manual was developed with the purpose of:

- providing practical guidelines for the persons responsible for the operation and maintenance of waste stabilisation pond systems,
- understanding typical failures experienced within the waste stabilisation pond system, and how to attend to and rectify such failures.

This operations and maintenance guide aims to emphasize important requirements as suggested by de Souza and Jack (2010), while also including the operation and maintenance requirements of duckweed-based stabilisation ponds.

B-2 Scope of manual

The scope of the manual will cover all aspects of the plant from the inlet works through to the final pond and sludge handling. A detailed breakdown of the scope of work is therefore as follows:

- Inlet works of water treatment plant
    - Removal of screenings
    - Handling and disposal of screenings
    - Training requirements
    - Health and safety aspects including training and equipment requirements

- Anaerobic digestors or ponds
    - Management of anaerobic pond systems
    - Cleaning of surface material
    - Pond cleaning requirements
    - Training requirements
    - Health and safety aspects

- Duckweed-based and algal-based waste stabilization ponds
    - Harvesting requirements
    - Maintenance and frequency of cleaning
    - Training requirements
    - Health and safety requirements

- Disinfection
    - Chlorine dosing control and retention time
    - Monitoring requirements
    - Training requirements
    - Health and safety requirements

- Estate management
    - Fence inspection and maintenance
    - Grass cutting
    - Security

- Sampling and monitoring
    - Sampling requirements in terms of the plant license

B-3 Inlet works

The inlet works of the treatment plant is critical to protect the rest of the plant against sand, grit, rags and other foreign material that may hamper the operation of the rest of the plant. It usually consists of some form of screening device (mostly a simple bar screen at pond systems) to remove rags, bags and other unwanted objects and grit channels, designed to slow the flow down to allow time for grit and sand to settle in these channels, thus removing it from the influent to the plant.

B-3.1 Operations requirements

Effective operation of the inlet works is the most important aspect of pond based plant operations. If foreign matter such as rags and grit are not removed from the influent as far as possible blockages and build-up of sand in the ponds is a given.

B-3.1.1 Screenings

The following operations and maintenance activities are suggested for the removal and handling of screenings from the bar screen:

- Screenings should be raked from the screen on a daily basis. They should be allowed to drain before disposal. A rake must be provided to the operator for this purpose.
- The screenings should be temporarily stored in a bin situated at the inlet works. The screenings should be sprinkled with chlorine of lime to prevent odours and flies.
- An area on site should be identified for the burial of the screenings. This should be far from the units of the treatment system, but still within the fence boundary.
- A trench should be dug and the screenings buried on a daily basis.
- Growth of weed should be controlled to prevent veld fires and discourage rodents.
- Implementation of groundwater and soil monitoring programs may be necessary, especially for larger sites. The cost of this should be carefully considered, and if not feasible then screenings should be disposed of on landfill.

B-3.1.2 Grit removal

Raw sewage typically enters a short rectangular channel to allow settling of grit. Grit is fine, solid particulate material such as sand or ash that enters the sewer. It has a relatively high settling velocity and once settled, is difficult to re-suspend. It normally accumulates in the first pond, close to the inlet works if not effectively removed.

Figure B-1: Typical bar screen (left) and grit channels (right)

Grit should be removed during low flow periods to avoid transport along the channel when disturbed.

- Grit should be removed daily. If allowed to accumulate, the grit will be washed into the pond during high peak flow.
- After isolating the channel or diverting the flow, scrape the grit off the channel with a spade and put it in a wheelbarrow.
- Dispose grit at a proper landfill site that has been indicated by a supervisor, which may be the same trench that is used for the disposal of screenings.
- The waste should be buried every day, if disposed of in a trench.

Another function typically associated with inlet works is flow measurement. An inlet flume will control the flow velocity and reduce the risk of grit entering the pond. Installation of a flow meter will provide accurate information on the flow. Alternatively, the operator should measure and record the water depth regularly. A graded strip, indicating the flow at any level, can be permanently fixed to the side of the channel. It will allow the operator to read and record the flow instantly. This strip can be fabricated based on the actual dimensions of the measuring flume. Recording the data regularly will provide important information necessary for future planning. Future planning includes gaining permission for new connections, increasing the size of the WSP, deciding whether it is still the best technology, renewal of licences, etc.

B-3.1.3 Summary of equipment requirements

Based on the operational functions described above, the following equipment is required for inlet works operation:

- Rake for screenings removal
- Bin for temporary storage of screenings
- Chlorine of lime for screenings dosing
- Modified spade for removal of grit from the channels and a spade for digging of disposal trench, if required.
- Wheel barrow for removal of grit to disposal trench

B-3.1.4 Health and safety considerations

The operator will be exposed to hazardous conditions during the operation of the inlet works and it is important that health and safety procedures be put in place. The operator must be provided with eye protection, elbow length thick rubber gloves, gum boots, masks and overalls for work on the inlet works. The operator must be trained in the correct use of

the safety equipment. It is important that the gloves be worn at all times when handling the screenings, as they will contain potentially harmful pathogens.

Chlorine of lime is a dry powder with bleaching properties. It is used as disinfectant. It is an irritant and inhalation of the dust and contact in the eyes should be avoided. A dust mask and eye protection should be used when handling it.

Ablution facilities must be provided for the operator, including a flushing toilet and hand basin, with toilet paper and clean hand towels. It is important that disinfecting hand soap be available at all times and the operator should be trained in good hygiene practices. He or she should wash their hands after working on the inlet works.

The operators should be vaccinated against hepatitis B. Regular de-worming is also required.

B-4 Anaerobic ponds

The main purpose of anaerobic ponds is to provide pre-treatment as they remove organic loads and settled solids, thus minimizing the amount of sludge that enters the primary stabilisation ponds. The retention time is short, that is, 3 to 5 days at temperature greater than 20°C (Mara, 1998) and depth between 2-4 m. The colour of the wastewater contained within the pond is normally dark brown to black, and the ponds normally contain no significant algal growth. A scum layer may be found on the surface of the ponds. The wastewater received in the anaerobic ponds typically has organic loads in the excess of 100 g BOD/m^3.day (more than 3000 kg/ha.day at for a depth of 3 m) (Kayombo et al. 2005).
As a complete process, anaerobic ponds serve to:

- Separate solids from dissolved material, as solids settle to the bottom as sludge
- Break down biodegradable organic material, releasing organic material into solution
- Store undigested material and non-degradable solids as bottom sludge
- Allow partially treated effluent to pass out

BOD removal in anaerobic ponds is accomplished by means of sedimentation of solids, followed by anaerobic digestion in the sludge formed by the settled solids. Anaerobic digestion is temperature dependent and is more intense above 15^{o}C. Anaerobic bacteria are sensitive to pH below 6.2 and acidic wastewater must therefore be neutralized prior to entering the anaerobic ponds. Anaerobic ponds, when properly designed, can achieve up to 40% removal of BOD at 10^{o}C, and 60% at 20^{o}C.

The installation of anaerobic ponds or a primary sludge anaerobic digestor is important for a combined duckweed-algal-based system. The usual practice of a facultative anaerobic first pond in the absence of anaerobic ponds or digestors may result in the conversion of ammonia nitrogen to nitrate by nitrifying bacteria and algae in the pond. As duckweed preferentially utilize ammonia nitrogen, it is preferred that the ammonia-rich effluent from the anaerobic ponds is treated directly with a duckweed pond directly after the anaerobic ponds.

Table B-1 below depicts the design parameters prescribed by several sources, each related to a specific loading rate and sewage characteristic.

Table B-1: Range of loading rates for anaerobic ponds from various sources

Reference	Retention time (days)	Listed Loading Rate	Converted Loading Rate (kg/m^3-day)	Depth (m)	Comments
Barnes, Bliss, et al. (1981)	8 to 40	25 to 40 grams/m^2 day (3.75 m depth)	0.007 to 0.011	2.5 to 5.0	Primarily for medium strength domestic sewage
Metcalf and Eddy (1979)	5 to 50	200 to 500 kg/ha-day (3.75 m depth)	0.005 to 0.015	2.5 to 5.0	Primarily for medium strength domestic sewage
Eckenfelder (1980)	5 to 50	250 to 4000 lbs BOD per acre-day (11.5 ft)	0.008 to 0.130	2.4 to 4.6	Broad range for all applications
Corbitt (1989)	1 to 50	0.05 to 0.25 kg/m^3-day	0.05 to 0.25	2.4 to 6.1	Loading "widely varying due to wastewater characteristics

B-4.1 Operations requirements

The key to keeping the anaerobic ponds healthy is the inlet works. If the inlet works operates effectively, little or no maintenance is needed at the anaerobic ponds. Should some grit enter the first anaerobic pond and accumulate in it the pond will become shallower and its retention time shorter. The pond will then have to be drained and the grit removed.

At some point it will be necessary to waste excess sludge, and drying beds should be installed for the management of this sludge. Alternatively, a tanker can be used to dispose of

the stabilized sludge on a landfill, provided that it has been classified and found appropriate for disposal according to the sludge guidelines. The expected volume of sludge is small.

All manholes and boxes should be kept clean and free from sludge. The operator should periodically spray these with clean water to prevent a build-up of sludge. Any blockages should be reported to the maintenance team immediately.

The scum on the surface of anaerobic ponds should be sprayed on a weekly basis but should not be removed as it aids the treatment process. In the event fly breeding is detected this material should be sprayed with clean water.

Weeds growing around this area should be removed.

B-4.1.1 Health and safety considerations

The health and safety considerations that apply to work at the inlet works also apply to the anaerobic ponds. Operators should wear gloves, safety boots, overalls and if necessary a mask when handling sludge and cleaning reactors. Operators must be trained to wash their hands after doing work on the anaerobic ponds.

The anaerobic ponds pose a drowning risk to animals and personnel. Unauthorized entry to the area should be prohibited and controlled and warning signs must be erected around the ponds.

B-5 Stabilisation ponds

If there is no anaerobic pond or digestor, anaerobic conditions will prevail in the first part of the initial pond. Digestion of organic material will take place, and methane will be produced as the biodegradable organic material decays. At the same time, inorganic substances such as ammonia-nitrogen and ortho-phosphorous will be released as a result of mineralization. The process is temperature sensitive. No algae or duckweed are expected on the first half of the pond as a result of inhibition by digestion by-products. Ammonia-nitrogen only becomes toxic to algae at concentrations exceeding 60 mg/l (Nhapi et al., 2003). As the soluble organic material, including fermentation products are degraded, algae or duckweed may be established.

B-5.1 Algal-based WSP systems

Algal-based systems are dependent on a number of factors, available light being one. Algae have a short doubling time and will multiply quickly. However, as the algae grow, their

concentration increases, resulting in an increased turbidity. Less light is able to penetrate and the growth of algae is limited. In this way, equilibrium is quickly established. Sufficient light is also required for bacterial destruction. Removal of faecal coliforms is effective in an algae-based WSP system. Algal cells are small and are capable of controlling their buoyancy. The cells are therefore uniformly suspended throughout the water column. During photosynthesis, oxygen is produced. This has a number of consequences. Firstly, the oxygen supports the growth of heterotrophic bacteria which are responsible for the metabolism of the organic substances in the water and actively reduce the COD. The algal cells and the bacteria serve as food for protozoa. This allows for the establishment of a variety of higher forms of animals, all sustained by the dissolved oxygen. This is an important factor to consider for the removal of faecal coliforms.

A disadvantage is that the algal cells remain in suspension and escape in the effluent. Their presence results in high COD and suspended solids concentrations, often exceeding the general standards. This is one reason why WSP systems seldom comply (Jack et al., 2006).

B-5.2 Duckweed-based WSP systems

Duckweed-based WSP systems have a distinctive floating mat of duckweed covering the surface of the pond. It has been demonstrated that these systems are able to remove COD and nutrients effectively. Since algal growth is inhibited, the effluent is free from suspended material and therefore has a lower COD and suspended solids concentration compared to algae-based WSP systems.

The disadvantage is that production of oxygen is limited to the surface layer associated with the mat of duckweed. The water column remains essentially anaerobic. Higher life-forms such as protozoa and their predators can therefore not be established. The important mechanism of grazing on bacteria is absent, thereby reducing the efficiency of faecal coliform removal. This explains why it appears that the ponds are under designed with respect to faecal coliform removal.

B-5.3 Combined duckweed-algal-based pond system

It is clear from the discussions above that both algal systems and duckweed systems present various advantages and disadvantages. The first ponds should be operated with a floating duckweed cover, by modifying the overflow weirs to retain surface material, but it is recommended that the subsequent ponds should be operated as algal-based ponds. Algal populations will establish rapidly if the overflow weirs are designed to encourage wash-out of surface material.

B-5.4 Operations requirements

The stabilization ponds have the following maintenance requirements:

1. If the system is operated without an anaerobic pond system or digestor, with the consequence that no sludge is removed from the system prior to the stabilisation ponds, the residence time in the first pond will gradually be reduced and anaerobic conditions will be observed in the second pond after a few years. When that happens, the first pond may be isolated and cleaned. The influent can be temporarily diverted to another pond that is not being desludged. Removed sludge should be disposed at an appropriate landfill sites indicated by the supervisor.
2. Ideally the duckweed should be harvested at a continuous rate to remove nutrients from the system. Enough duckweed should be removed on a daily basis so that by the following day a continuous lawn has once again established. This will be a trial and error process that will vary with temperature. The surface density of the duckweed is important. If the density is too high, duckweed plants will have limited access to nutrients and limited light, gas exchange, and space to grow resulting in lower growth rates and poor nutrient uptake. However, the density must be high enough to prevent light penetration to the water to ensure that algal growth is inhibited. An optimal mat density is approximately 45 g-dry.m^{-2} (750 g-wet.m^{-2}).
3. Existing pond systems are not designed for harvesting, so provision should be made to modify these ponds in such a way as to make harvesting possible. If no provision is made for harvesting, the ponds will be unlikely to comply with the nutrient removal requirements as a portion of the nutrients assimilated by the duckweed will be returned to solution upon their anaerobic degradation in the sediment. Regular monitoring will indicate failing conditions. It is expected that the uptake of nitrogen by duckweed will range from between approximately 1.2 kg N/ha.d to 4 kg N/ha.d, and the uptake of total phosphorus from 0.2 kg P/ha.d to 1 kg P/ha.d, depending on the season and temperature.
4. The nitrogen concentration of the effluent from the anaerobic digestor as well as duckweed-based ponds where it enters the algal-based ponds should be monitored, as it is an indication of the performance of the duckweed ponds and will be useful for determining the required harvesting rate. Up to 98% removal of ammonia nitrogen can be achieved in a well-functioning duckweed system at warm temperatures. Poor removal efficiency is an indication of an incorrect harvesting rate, or of growth limiting conditions. The operator should therefore be provided with a means to measure nitrogen, or samples should be regularly sent away for analysis.
5. Depending on the duckweed species, the length of the roots and size of the fronds may be an indication of when harvesting should take place. *Lemna* spp. display

increased root length and frond size under nutrient limiting conditions. If this is observed then the harvesting frequency should be increased. The effect of nutrient limiting conditions on frond size and root length is illustrated in Figure B-2 and Figure B-3.

6. Any foreign floating material that may have bypassed the screens or blown into the ponds should be removed daily.
7. Any accumulated solids in the pond's inlets and outlets should be removed daily.
8. Embankment maintenance:
    - Repair of any damage to the embankments caused by rodents or other animals.
    - Repair of any damage to the embankments caused by erosion.
    - Grass and weeds should be removed on the embankment. Grass growing on the embankment may cause cement embankment to crack or break and accelerate erosion in the wave zone and finally structural damage.
    - Remove the grass on the cement embankment with a spade. Try to remove as much of the root system as possible to avoid re-growth.
    - Proper control of the grass/weed will prevent the need for disposal of huge quantities of grass/weeds. If required, the biomass should be disposed of at a suitable dumping site that has been indicated by a supervisor. This could be the same trench that is used for the burial of screenings.
9. Remove settled solids at the inlet because they affect the flow pattern and thereof mixing within the pond. Septic conditions and odors may be caused under these conditions.

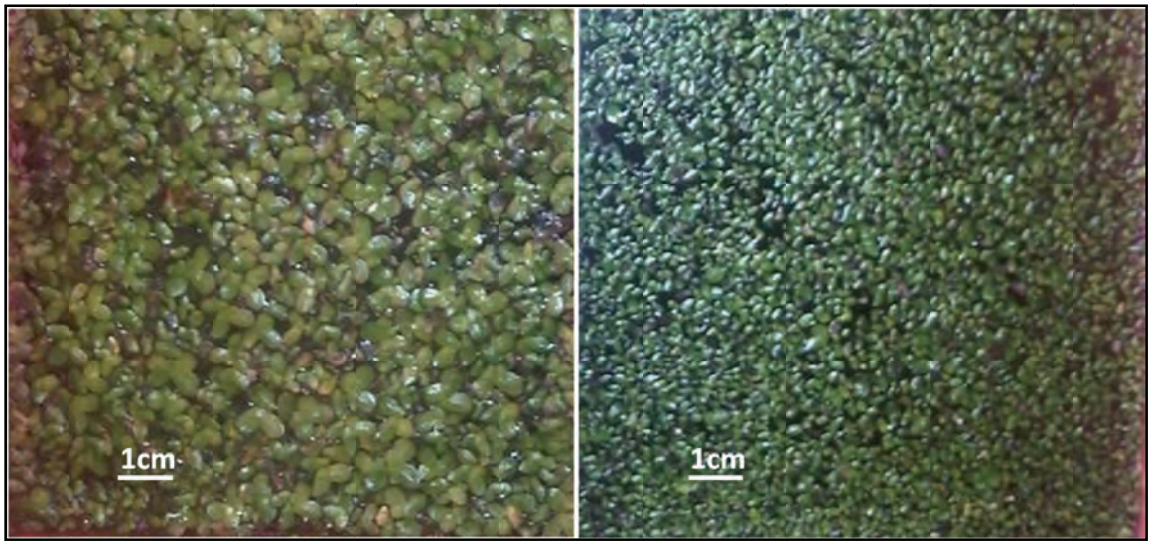


Figure B-2: Frond size of Lemna spp grown in low (left) and high (right) nutrient concentrations

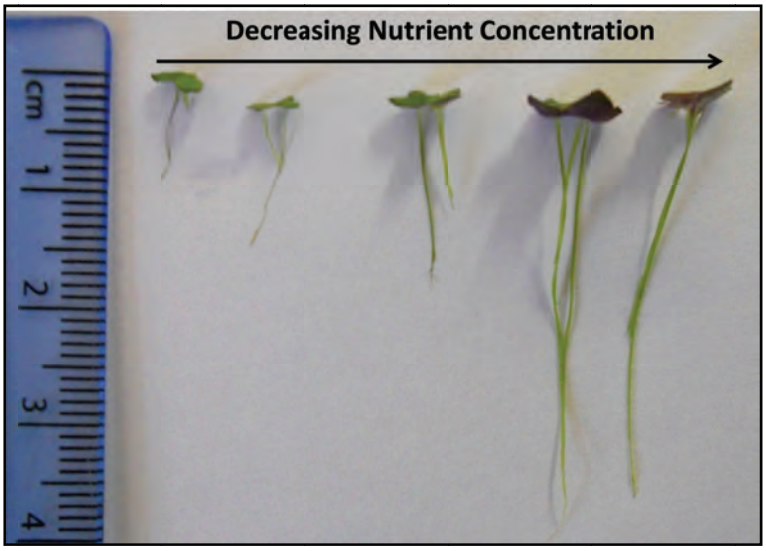


Figure B-3: Increasing root length of *Lemna* spp. with decreasing nutrient concentration

B-5.5 Health and safety considerations

The health and safety considerations that apply to work at the inlet works also apply to the stabilisation ponds, particularly the primary facultative ponds. Operators should wear gloves, safety boots, overalls and if necessary a mask when handling scum and de-sludging the ponds. Operators must be trained to wash their hands after doing work on the stabilization ponds.

B-6 Disinfection

The chlorine dispenser should be filled with either calcium hypochlorite granules (HTH) or a solution of sodium hypochlorite, and the resulting concentrated solution dosed into the final effluent stream. Optimum disinfection with chlorine occurs at pH values between 5 and 7. It is not possible to predict the required dosage. A combined chlorine residual concentration of 0.5 should be maintained by adjusting the dosing rate based on the flow rate. An indirect method to determine the optimum dosing rate is to record the free residual and combined chlorine whenever a sample is collected for microbiological analysis and to adjust the chlorine dosing to meet a set combined free residual concentration accordingly. It is expected that the required optimum dosage will vary with seasonal changes. For example, higher ammonia-nitrogen concentrations are expected during the colder winter months.

B-6.1 Health and safety considerations

Both calcium and sodium hypochlorite are strong oxidizing agents, and the necessary protective clothing must be worn when handling these chemicals. Gloves, eye protection, dust mask and overalls are mandatory. Operators must be trained in the correct handling of chlorine chemicals.

B-7 Estate maintenance and public safety

The site area should be kept neat at all times. The grass should be cut regularly, especially in summer. The empty ponds should be kept free from vegetation. No rubbish should be dumped on the site, and the trench dug for disposal of screenings and grit should be covered adequately.

The WSP system area has been fenced to prevent public access. The fence should be inspected on a regular basis and any damage to external fences and gates or points of access to the system should be repaired immediately.

Animals and unauthorised people should be kept out of the ponds site. A visitor's record sheet may be provided for any person entering the site. No animals should be deliberately kept on-site for grazing and drinking.

A "No entry" sign should be mounted on the fence or gate.

B-8 Final effluent monitoring

The monitoring requirements for a pond system will likely be specified in the water use license for the system, which may vary according to the sensitivity of the river system into which the water is discharged. However, it is expected that the plants will need to be monitored and compared to at least the DWA General Authorisation limits for discharge.

Monitoring of the final effluent of a waste stabilisation ponds system is required to address the following needs:

- Detect whether or not the effluent complies with the local discharge or reuse standards,
- Detect any sudden failure, or determining if the pond effluent has started to deteriorate and
- Identify the cause of the problem and the remedial actions to be taken.

As per General Authorisation, 2004, wastewater monitoring in waste stabilisation ponds should be performed by grab sampling. Grab sampling is when one sample (i.e. final effluent sample) is taken at a specific time. A grab sample reflects performance only at the point in time that the sample was collected, and then only if the sample was properly collected. Details on how to conduct monitoring are provided below.

B-8.1 Sampling equipment

The following equipment is required for monitoring:

- Two types of sampling bottles;
  - clean 1 litre bottles for physical and chemical analysis
  - sterilised bottled for bacteriological analysis (do not rinse)
- A scoop to collect water from the outlet
- A cooler bag/box with ice
- Watch
- Thermometer
- Pen and log book to record observations

B-8.2 Record sheet

A record sheet should be filled in that includes the following information:

- Date and time when the samples were collected
- Both environmental and water temperature
- Indicate from which pond/s is/are the sample/s collected
- Write a code on the bottle to identify the system from which the sample/s was/were collected.

B-8.3 After sampling

- Keep the sample bottles cool by putting them in the cooler bag/box with ice.
- Take the samples to the nearest (preferably accredited or DWAF approved) laboratory for analysis preferably within 6 hours maximum (for microbiological analysis) and 12 hours maximum (for physical and chemical) from the time of collection.
- Parameters to be analysed for if discharging and or used for irrigation are shown in the following tables.

Strictly speaking waste stabilisation pond systems are designed not to discharge to the environment (and in particular streams/rivers), and this is verified by the conditions attached to *Permissible Utilisation and Disposable of Treated Sewage Effluent*. A DWA license/permit/exemption should be obtained by the municipality or any person/industry for the operation of waste stabilisation ponds system.

If the waste stabilisation ponds system is discharging or final effluent re-used, the final effluent results should be compared to the DWA General Authorisation as shown in the tables below.

Table B-2: Wastewater limit values applicable to discharge of wastewater into a water resource

Substance/parameter	General limit
Faecal coliforms	1000/100 ml
Chemical oxygen demand (COD)	75 mg/l (after removal of algae)
pH	
Ammonia (ionized and unionized) as Nitrogen	6 mg/l
Nitrate/Nitrite as Nitrogen	15 mg/l
Chlorine as free chlorine	0.25 mg/l
Suspended solids	25 mg/l
Electrical conductivity	70 mS/m
Orthophosphate as phosphorus	10 mg/l
Fluoride	1 mg/l
Soap, oil or grease	2.5 mg/l

Irrigation with waste stabilisation ponds effluent should only be practiced in a manner indicated by the supervisor. Conditions under which waste stabilisation ponds effluent could be used for irrigation are set out in Appendix D of the Guide for Management of Waste Stabilisation Ponds.

Table B-3: Wastewater limit values applicable to irrigate with wastewater

Determinant	Quality
Electrical conductivity	<200 mS/m
pH	6-9
Chemical oxygen demand (COD)	<400 mg/l
Faecal coliforms	<100 000/100 ml
Sodium absorption ratio (SAR)	<5 for biodegradable industrial waste water

If it is noted by the operator or supervisor that a discharge of the final effluent is occurring, the effluent water quality must be monitored. An application for an amendment to the current water license must be lodged immediately with the local authority. If discharge is not permitted than it may be necessary to increase the capacity of the system.

B-9 Management and operations control

No new connections should be allowed without an assessment of the impact on the flow and load.

It is important that the plant is regularly inspected to ensure that necessary maintenance can be carried out when necessary.

Training of the operators is important, and on the job assessments should be conducted regularly to ensure that the correct procedures are being adhered to. Operators should be provided with a record sheet to quickly identify issues of concern. A list of suggested activities that can be considered for inclusion in an operational checklist is presented in Table C-1 in Appendix C.

B-10 Budget considerations

The following items should be considered when the budget for the WSP system is prepared. This will ensure that sufficient funds are available for the operation and maintenance of the plant in a safe and compliant manner.

Table B-4: Suggested budget items for consideration

Variable Costs	**Maintenance**
Chemicals	Civil Structure %
1. Disinfectant	Electrical Equipment
Electricity	Mechanical Equipment
Potable Water	Instrumentation Equipment
Waste Disposal	Equipment Spares
1. Grit Removal	Estate Management
2. Screening Removal	Building Maintenance
3. Digested sludge Disposal	Preventative Maintenance
	Tools
Manpower	**General**
Bonuses	Cleaning Materials
Housing Subsidies	Consumables
Leave Pay	Health & Safety
Long Service Award	Professional Services (Contractors)
Overtime / Standby	Security
Protective Clothing	
Operator Salaries	

B-11 References

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Appendix C: Operational Checklist

Table C-1: Suggested items for inclusion in an operational checklist, including transfer pumps stations.

Operation and Maintenance	**Frequency**						
	Daily	**Weekly**	**Monthly**	**3 monthly**	**6 monthly**	**Yearly**	**As needed**
Plant Survey							
Take note of the following conditions:							
Any buildup of scum on pond surface and discharge outlet boxes		x					
Signs of burrowing animals	x						
Anaerobic conditions: noted by odour and black colour, floating sludge, large number of gas bubbles	x						
Scum/foam on anaerobic ponds		x					
Water grown weeds		x					
Evidence of embankment erosion		x					
Overgrown vegetation requiring attention		x					
Fence damage		x					
Evidence of short circuiting		x					
Water level in ponds		x					
A review of the information obtained from the observations should be included in the next year's planning activities							
Plan, schedule and correct problems found		x	x			x	

Pre-treatment							
Clean screens and dispose of screenings correctly	x						
Remove grit from grit channels		X					
Check inlet flow meter		X					
If discharge is once or twice per year, the discharge permit may require observations of the following:							
Odour		X					
Aquatic plant coverage of pond		X					
pond depth			x				
Flow (influent)	x						
Rainfall	x						
If discharge is continuous, the discharge permit may require the following information:							
Weather	x						
Flow (influent)	x						
Condition of all ponds	x						
Pond effluent quality (specified in permit)		X					
Other tests and frequency will be defined in the permit							

Mechanical equipment							
Check mechanical equipment and perform scheduled preventive maintenance on the following pieces of equipment according to the manufacturer's recommendations:							
Pump stations:							
Remove debris	x						
Check pump operation	x						
Log running times	x						
Lubricate		X					
Chlorinators							
Check feed rate	x						
Check supply							x
Flow measuring devices							
Verify accuracy, calibrate if necessary		X					
Valves and gates							
Check to see if set correctly	x						
Open and close to be sure they are operational		X					

Appendix D: Draft Training Guide

As with the Operations and Maintenance Guide, this guide is a draft and will be finalized once the pilot scale trials have been conducted in the next phase of the project.

D-1 Operator qualifications

Is it recommended that the operator or process controller responsible for the plant be a Class 1 operator, with some experience, alternatively a Class 0 operator will suffice provided that prior learning is applicable to water treatment. General workers may be required at larger sites to assist with maintenance work. All operators must be given training specific to the management of waste stabilization pond systems, and combined duckweed-algae based systems in particular.

D-2 Training overview

The following objectives should be achieved when training operators for waste stabilization pond systems:

- The operators should have a good understanding of the process and the goals of treatment, including the standards of compliance
- There should be a good understanding of the required health and safety aspects, and how to ensure compliance for personal and public safety.
- The importance of inspection and reporting must be emphasized through the development of checklists.
- Maintenance considerations must be highlighted and supported by preventative checklists and maintenance schedules.
- Specific training should be given on the growth and management of a plant based system (duckweed and algae) versus a bacteriological system such as is found in activated sludge plants.
- Monitoring requirements must be understood, both in terms of the legal monitoring and reporting requirements, as well as the operational monitoring required. The operator must be trained to react correctly to the information gained from operational monitoring and should understand process troubleshooting.

D-3 Combined duckweed- algae based waste stabilization pond process knowledge

Before any operational knowledge is transferred, the operators must first be trained in the fundamental principles of the duckweed- algae based pond systems in order to gain an understanding of the system as a whole. This will include the following:

- The composition of primary effluent that is fed to the plant, and the need to remove COD, nitrogen and phosphorus from the waste water, while reducing pathogens.
- The importance and function of the inlet works.
- The application of anaerobic digestion for the breakdown of complex organic matter into simpler soluble nutrients which are easily taken up by algae and duckweed.
- The principles of duckweed-based systems, and how they treat the water, through nutrient uptake into the plant biomass and COD removal through anaerobic processes, as well as removal of some pathogens by sedimentation. The importance and role of harvesting must be emphasized.
- The principles of algal-based systems and how they treat the water, through further nutrient removal by algal uptake, and pathogen destruction through sunlight exposure and further time for sedimentation.
- The advantages and disadvantages of duckweed and algal-based systems, and why the combination of the two systems results in improved final effluent quality.
- The role of the final duckweed pond in effluent polishing.
- Fundamentals of disinfection and when it will be required, as well as how to control the dosing to meet the standards for pathogen removal.
- The final effluent standards that can be achieved with this system if it is functioning correctly.

D-4 Specific operational training

D-4.1 Inlet works

The operators must trained theoretically as well as through “on the job” practical training how to manage the inlet works correctly. This will include:

- Tools required, and the correct maintenance and cleaning of tools
- Correct method for raking of the screens and disposal of screenings
- Correct method for the removal of sand from grit channels and the correct disposal of the grit
- Health and safety requirements
- Correct use of on-site ablution facilities, and the importance of personal hygiene

D-4.2 Anaerobic digestor

Practical training should be given to the operators on the following aspects of the anaerobic digestor or pond:

- Start up of new digestor systems
- De-sludging requirements, including frequency and method
- Management of drying beds, if used, and correct disposal of sludge
- Cleaning of manholes, scum boxes and splitter boxes to prevent blockages
- Trouble-shooting of digestor process and identification of system failure
- Health and safety requirements

D-4.3 Duckweed ponds

Practical training on the management of the duckweed ponds should include:

- Cleaning of inflow and outflow weirs to prevent blockages and short circuiting
- Removal of foreign floating material such as papers or plastic and correct disposal of these
- Harvesting method and frequency, as well as drying and handling of the harvested plants
- Maintenance of the correct surface density by controlling the harvesting rate
- Ability to identify when the plants are stressed, as well as when they are nutrient limited by observing root length and frond size
- Health and safety requirements

D-4.4 Algal ponds

Practical training should include:

- Cleaning of inflow and outflow weirs to prevent blockages
- Removal of foreign floating material such as papers or plastic and correct disposal of these.
- Health and safety requirements

D-4.5 Final duckweed effluent polishing pond

- Cleaning of inflow and outflow weirs to prevent blockages and short circuiting
- Removal of foreign floating material such as papers or plastic and correct disposal of these.
- Harvesting method and frequency, as well as drying and handling of the harvested plants.
- Maintenance of the correct surface density by controlling the harvesting rate.

- Ability to identify when the plants are stressed, as well as when they are nutrient limited by observing root length and frond size.
- Health and safety requirements

D-4.6 Effluent

Practical training should be given to the operators on the following aspects of the final effluent:

- Disinfection requirements and dosing
- Health and safety aspects

D-5 Monitoring requirements

Both operational and regulatory monitoring will be required. Regulatory monitoring will only require the sampling of the final effluent, and reporting to the relevant authority. Operators should be trained in the correct method of sampling so as to avoid contamination, and must understand how to preserve and transport samples.

Operational monitoring is important for the management of the duckweed system. This will involve sampling of the effluent of the anaerobic digestor in order to determine the concentrations of ammonia nitrogen, COD and total phosphorus entering the duckweed pond. The effluent of the duckweed ponds must be monitored specifically for total nitrogen, as removal rate of nitrogen is an indication of the correct functioning of the duckweed ponds, and will affect the required harvesting rate. As with regulatory monitoring, the operators should be trained in the correct methods of sampling and sample handling. If possible a facility should be provided on-site for nitrogen measurement, and the operator should be trained in the use of the equipment. If no facility is available samples should be sent away to a laboratory at least on a monthly basis.

D-6 Inspection and reporting

Operators should be trained in the correct method of plant inspection and reporting of faults. Site specific operations checklists should be prepared which are completed by the operator on either a daily, weekly, or monthly basis. These should include tasks completed as well as observations for problem diagnosis. These checklists will enable the analysis of trends to assist with future planning. The operator should be trained to identify problems based on issues noted in the checklist, and should report any problems immediately.

D-7 Maintenance

As with the operations inspections, maintenance inspections should be conducted regularly by the operator, and site specific checklists should be prepared for this purpose. Preventative maintenance should be conducted according to a set schedule.

General workers should be trained in specific maintenance tasks, including:

- Grounds maintenance, including grass cutting and weed and rubbish removal
- Maintenance of embankments, including repair of damage by erosion or rodents and weed removal.
- Burial of screenings and grit, if disposed as such
- Sludge handling
- Handling of dried duckweed material
- Health and safety considerations