

Bio-Recovery of N and P from an Anaerobic Digester Effluent: The Potential of Duckweed (*Lemna minor*)

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Abstract

The possibility of growing duckweed (*Lemna minor*) on anaerobic digester effluent and its nitrogen and phosphorus acquisition potential was studied. Duckweed was cultured for 21 days on an anaerobic digester effluent using two methods: static aeration (SAT) and normal batch reactor (NBR) techniques, respectively. The treatments involved pure effluent and 1/10, 1/25 and 1/50 effluent dilutions, respectively. Fifty duckweed fronds were grown in the anaerobic digester effluent for 21 days. At the end of the growth period, phosphate levels, total ammonium nitrogen (TAN) and total oxidized nitrogen (TON) contents of the growth media were determined. Total nitrogen concentration and orthophosphate P content in the duckweed were also determined. The results obtained indicated that duckweed was capable of growing on the anaerobic digester effluent provided its TAN content did not exceed 42 mg N l⁻¹. Nitrogen uptake by the duckweed from the effluent ranged between 53 and 115.7 mg l⁻¹ whereas P uptake varied from 1.40 to 8.4 mg P l⁻¹. The relative growth rate of duckweed in the anaerobic digester effluent was observed to be comparable to literature value of 0.22 mg day⁻¹. The results have demonstrated that duckweed has the potential to recover N and P from anaerobic digester effluent.

Introduction

Waste management is a serious environmental problem facing governments in many developing countries in recent years. Solid waste forms a major component of the overall waste generated in these countries. The conventional methods of municipal solid waste management involving collection and disposal by either incineration or landfill have inherent technical and economic drawbacks, which render them ineffective and unsustainable. The ineffectiveness of contemporary municipal solid waste management has culminated in a number of health and environmental problems such as cholera outbreak and water pollution (Iqbal, 1999). Therefore, the need has arisen for research to be conducted to establish more effective, sustainable and environmentally friendly systems of waste management.

A combination of anaerobic pre-treatment followed by photosynthetic post-treatment may have the potential for effective recovery of nutrients from solid waste. This technique is based on the fact that the main nutrient assimilating capacity is housed in photosynthetic plants. The use of aquatic macrophytes such as duckweed in waste water treatment has attracted global attention in recent years (Van der Steen *et al.*, 1999). Duckweed (*Lemna minor*) has been applied on the surface of stabilization ponds and has contributed to nutrient recovery from waste water. Duckweed can accumulate considerable amount of nutrients, which can be removed by simple low-cost harvesting technologies. The harvested duckweed may be used as a valuable feed source for fish and livestock (Skillicorn *et al.*, 1993).

Duckweed is adaptable to a wide variety of geographic and climatic zones. It grows on polluted water, saline water and eutrophic water bodies (Oron *et al.*, 1986) and grows rapidly. Compared to other aquatic plants, duckweed is less sensitive to pH fluctuations, pest and disease damage and has a high nutrient recovery capacity (Landolt & Kandeler, 1987). Moreover, duckweed is adaptable to different sources of waste material such as biogas effluent, animal manure, urban refuse and kitchen waste (Gijzen & Khonder, 1997), thereby, making it potentially suitable species for nutrient recovery in an anaerobic digester effluent.

An important advantage of duckweed-based systems of waste treatment is that nutrients are partly recovered rather than being lost to the atmosphere, or removed with the effluent as in other

systems of waste management. The proposed anaerobic-photosynthetic process is energy efficient, cost-effective and applicable to a variety of rural and urban conditions (Oron, 1994). The study was carried out to establish the possibility of growing duckweed on an anaerobic effluent from a digester and to assess its nitrogen and phosphorus recovery capacity.

Materials and methods

Anaerobic digester effluent

The anaerobic digester effluent was obtained from a full-scale anaerobic digester treatment waste under thermophilic conditions (55 °C) (Six & De Baere, 1992). The effluent was squeezed out as paste from the mixed solid waste, which was treated anaerobically. A summary of the effluent characteristics is presented in Table 1.

TABLE 1
Total ammonium nitrogen (TAN) and phosphorus contents, and pH of anaerobic digester effluents.

<i>Effluent</i>	<i>TAN (mg N l⁻¹)</i>	<i>Phosphorus mg P l⁻¹</i>	<i>pH</i>
Pure	260	9	8.1
1/10 dilution	202	6.3	8.0
1/25 dilution	64	2.4	7.9
1/50 dilution	42	1.4	7.7

A nutrient culture to be used as control growth medium was prepared following the nutrient culture composition prescribed by Teisseire & Vermaat (1999) and stored at 4 °C in a refrigerator. The composition of the nutrient culture is presented in Table 2.

TABLE 2
Chemical composition of nutrient culture (control)

<i>Nutrient</i>	<i>Concentration (mg l⁻¹)</i>
KNO ₃	202
KH ₂ PO ₄	50.3
K ₂ HPO ₄	27.8
K ₂ SO ₄	17.4
MgSO ₄ ·7H ₂ O	49.6
CaCl ₂	11.1
Na ₂ -EDTA	10.0
FeSO ₄ ·7H ₂ O	6.0
H ₃ BO ₃	5.72
MnCl ₂ ·4H ₂ O	2.82
Zn SO ₄ ·7H ₂ O	0.6
(NH ₄) ₆ Mo ₇ O ₂₄ ·4H ₂ O	0.043
CuCl ₂ ·2H ₂ O	0.002
CoCl ₂ ·6H ₂ O	0.054

Treatments

The duckweed was obtained from a stock solution at Ghent University, Belgium, which had been acclimatized to domestic waste water for 6 months. Each experimental unit comprised 50 healthy duckweed fronds (80 mg dry weight) grown in batch reactors (miniature ponds of 5.5 cm depth, 353.4 cm² surface area containing 1500 ml effluent or nutrient culture (control) (Fig. 1).

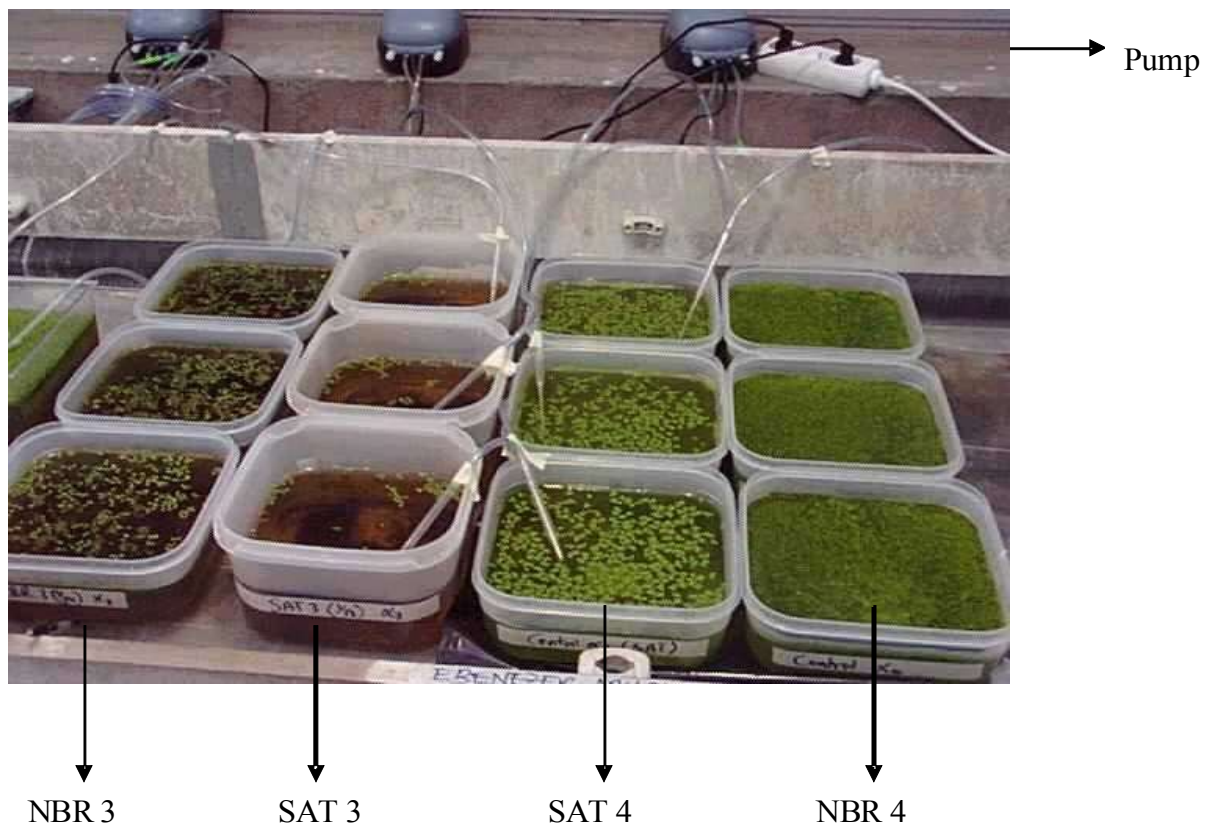


Fig. 1. Duckweed growing under aerobic (SAT) and anaerobic (NBR) conditions on effluent (3) and nutrient culture (4).

Two techniques were used to cultivate the duckweed fronds: (i) Static aeration technique (SAT): A method whereby duckweed cultivated in miniature ponds were exposed to oxygen supplied by pump. (ii) Normal batch reactor technique (NBR), in which duckweed fronds were grown under anaerobic conditions as described by Edwards (1990) (Fig. 1).

Each technique had four treatments and three replications. The treatments were:

Static aeration technique (SAT):

SAT 1: 1/10 dilution + aeration

SAT 2: 1/25 dilution + aeration

SAT 3: 1/50 dilution + aeration

SAT 4: nutrient solution + aeration (control)

Normal batch reactor technique (NBR):

NBR 1: 1/10 dilution without aeration

NBR 2: 1/25 dilution without aeration

NBR 3: 1/50 dilution without aeration

NBR 4: nutrient solution without aeration (control)

Total ammonium nitrogen (TAN) and total oxidized nitrogen (TON) contents of the effluents were determined at a regular interval of 7 days from day 1 of the experiment to the end of the experiment (21 days), using the Steam Kjeltic distillation method (Vandenabeele *et al.*, 1990). The ortho-phosphate-P ($\text{PO}_4^{3-}\text{-P}$) levels in the effluent were determined spectrophotometrically at 700 nm (APHA, 1992). The pH and ambient temperature of the media were measured using the pH meter and thermometer, respectively. The batch reactors were placed randomly under

metal halide lamps (Phillips HPIT) at a light intensity of 79.6 $\mu\text{E}/(\text{m}^2\text{s})$. Photosynthetically active radiation (PAR) was 16 h daylight and 8 h darkness. The average temperature was maintained at 25 °C and evaporation losses were compensated by adding deionised water once a day throughout the duration of the experiment.

Relative growth rate of duckweed

Relative growth rate (RGR) of duckweed was studied under laboratory scale batch experimental conditions. The RGR was determined from the biomass dry weight of the plant at the beginning of the experiment (DW_1) and at the end of the experiment (DW_{21}) using the equation (Hunt, 1978):

$$\text{RGR} = (\ln DW_{21} - \ln DW_1) / t,$$

where t is time in days. DW_1 was determined by weighing 50 fronds after drying at 105 °C at the beginning of the experiment while DW_{21} was obtained by drying the total biomass harvested at the end of 21 days to constant weight at 105 °C. Dry matter accumulated (biomass dry matter increment) was determined from $DW_{21} - DW_1$ using 50 fronds each, from the effluent and culture medium after 21 days.

Nitrogen and phosphorus contents of duckweed

Total nitrogen concentration in duckweed was determined after 21 days using $\text{H}_2\text{SO}_4/\text{H}_2\text{O}_2$ digestion and standard HCl titration using mixed bromocresol blue-methyl orange indicator (Royal Tropical Institute, 1984) and the orthophosphate-P content of the duckweed was also measured spectrophotometrically using ammonium molybdate in acidic medium (Royal Tropical Institute, 1984).

Results and discussion

Total ammonium-N concentration in effluent and duckweed growth

The experiment revealed that duckweed could neither survive on pure effluent nor on effluent dilutions of 1/10 and 1/25. (The effluent dilution was done with deionized water). Under both aerated and non-aerated conditions, the duckweed plants died between 3–7 days after planting. Total ammonium-N contents of the effluents ranged from 80 mg l^{-1} to 202 mg l^{-1} for 1/25 and 1/10 dilutions, respectively. These values were very high when compared to the reported values of 10 and 50 $\text{mg ammonia-N l}^{-1}$ in domestic and industrial waste waters (Veenstra *et al.*, 1995). However, the $\text{NH}_4\text{-N}$ content (260 mg N l^{-1}) of the pure effluent was comparable to the level reported by Veenstra *et al.* (1995).

In the literature, the effect of ammonia-N on the growth of plants has not been consistent. For instance, Clement & Merlin (1995) reported that total $\text{NH}_4\text{-N}$ in effluents consists of two principal forms: the ammonium ion (NH_4^+) and un-ionized ammonia (NH_3). They attributed ammonia toxicity to the effect of NH_3 only. However, other workers claim both forms of ammonia-N at high concentrations are toxic to plants (Litav & Lehrer, 1978; Monselise & Kost, 1993). In another study, Oron *et al.* (1984) found that total $\text{NH}_4\text{-N}$ level of 200 mg l^{-1} in domestic waste water was unfavourable to duckweed growth at pH 7 but Koles *et al.* (1987) reported the death of duckweed when the ammonium nitrogen concentration in the growth medium was above 50 mg l^{-1} .

According to Ingermasson *et al.* (1987), at pH above 8, ammonium is transformed into gaseous ammonia, which is toxic to duckweed. They explained that high NH_4^+ concentration could result in depolarization of the plant membrane, resulting in general inhibition of anion transport and growth of duckweed. Since duckweed could not survive on pure effluent and lower dilutions (1/10 and 1/25) during the experiment, all subsequent analyses were carried out only on the higher effluent dilution (1/50) and the control under both aerated and non-aerated conditions. The mean dry weights, the increment in biomass and the relative growth rate under experimental conditions are presented in Table 3.

TABLE 3
Effect of treatment on growth parameters of Duckweed

Treatment	Mean dry weight (mg) at day 0 (DW_0)	Mean dry weight (mg) at day 21 (DW_{21})	Increase in biomass (mg) after 21 days	Relative growth rate (RGR) d^{-1}
SAT 4	0.08 ± 0.01	7.91 ± 1.1	7.83 a	0.22 a
SAT 3	0.08 ± 0.01	0.92 ± 0.2	0.84 c	0.12 c
NBR 4	0.08 ± 0.01	2.64 ± 0.1	2.56 b	0.17 b
NBR 3	0.08 ± 0.01	1.63 ± 0.4	1.55 b	0.14 b

Figures in columns followed by different letters are significantly different at 5% level.

The highest increase in biomass dry matter of 7.83 mg was observed in SAT 4 and the least biomass increase of 0.84 mg was recorded in SAT 3 (Table 3). Differences in growth rates observed between the duckweed growing in nutrient culture (SAT 4) and effluent (NBR 3 and SAT 3) could be attributed to differences in nutrient balance in the growth medium. Higher increases in growth rate were observed in the nutrient media, which had an optimal nutrient balance. Gijzen & Khonder (1997) observed that the growth rate of common duckweed in different nutrient media compared with a standard mineral growth medium was considerably different. However, relative growth rates in NBR 4 and NDR 3 were not significantly different probably as a result of the large algal bloom observed in NBR 4. Apart from partially shading the duckweed, the algae could compete with duckweed for the nutrients in the growth medium, resulting in poor growth and subsequently reduced biomass.

Iqbal (1999) reported that light penetration in water and competition for nutrients, and space by algae could become a nuisance when the duckweed mat is incomplete due to disturbances or poor growth. The difference in biomass increase between SAT 4 and NBR 4 could also be attributed to aeration. Under anaerobic conditions ammonium-N appears to be the predominant form of N in the effluent. Despite being the preferred N source for duckweed plants (Porath & Pollock, 1982), it may become one of the parameters inhibiting the growth of duckweed in waste water (Lüönd, 1983). The growth rate of duckweed observed in the study appeared to be lower than the maximum relative growth rate values measured in lesser duckweed (*Lemna aquinoctialis*) and Indian duckweed (*Wolffia microscopica*) (Hillman & Culley, 1978).

Nitrogen uptake

Total ammonium-N (TAN) loss in the anaerobic digester following growth of duckweed is presented in Fig. 2. There was generally a decline in TAN content in both SAT 3 and NBR 3 over the growing period of 21 days. The initial TAN concentration of SAT 3 and NBR 3 were 64 mg N l⁻¹ and 61 mg N l⁻¹, respectively. By the end of the experiment, the TAN concentration had decreased considerably to 2 mg N l⁻¹ for SAT 3 and 0.8 mg N l⁻¹ for NBR 3. In the nutrient culture, total oxidized nitrogen (TON) instead of TAN was measured since TAN values were found to be insignificant compared to values recorded in the effluent. However, for purposes of

comparison, the recorded values in all cases were converted and reported in terms of nitrogen content per litre (N l^{-1}). TON concentration in effluent after 21 days of growing duckweed in the nutrient medium is presented in Fig. 3.

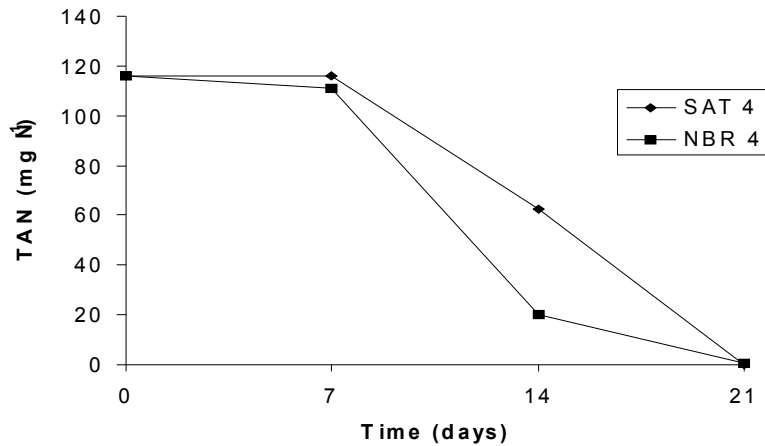


Fig. 2. Depletion of total ammonium-N (TAN) from growth media during growth of duckweed using static aeration (SAT) and normal batch reactor (NBR) techniques

Total TON concentration at the start of the experiment was 116 mg N l^{-1} for both SAT 4 and NBR 4. This amount decreased to 0.1 mg N l^{-1} and 0.3 mg N l^{-1} at the end of the experiment for SAT 4 and NBR 4, respectively. The decline in TAN and TON levels over the experimental period probably indicated a relatively high nitrogen uptake rate of duckweed.

Comparison of decline in TAN and TON concentrations in effluent and nutrient culture

TAN decline in the media following duckweed growth (Fig. 2) started from day 1 whereas a lag phase occurred in TON decline following duckweed growth (Fig. 3). It has been reported that approximately 50% of the total N load is assimilated by duckweed while the remaining N is removed by indirect processes other than plant uptake, such as denitrification and volatilization of ammonia (Körner & Vermaat, 1998). A further study to investigate the extent of nitrogen losses via denitrification and volatilization is, therefore, suggested.

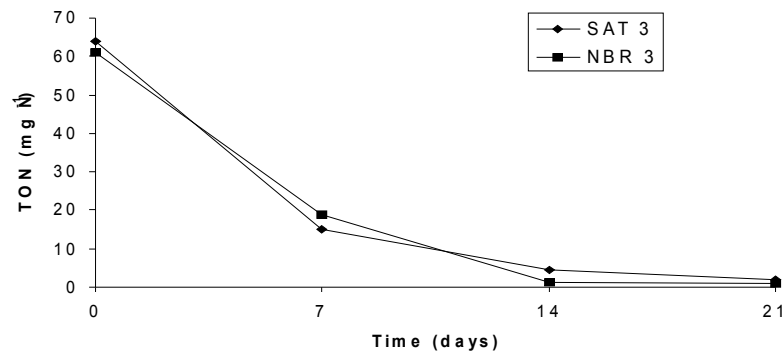


Fig. 3. Depletion of total oxidized nitrogen (TON) from growth media with time during growth of duckweed using static aeration (SAT) and normal batch reactor (NBR) techniques

Phosphorus concentration in effluent and nutrient media following duckweed growth

Reduction in P content in SAT 3 and NBR 3 and for SAT 4 and NBR 4 following growth of duckweed are presented in Fig. 4.

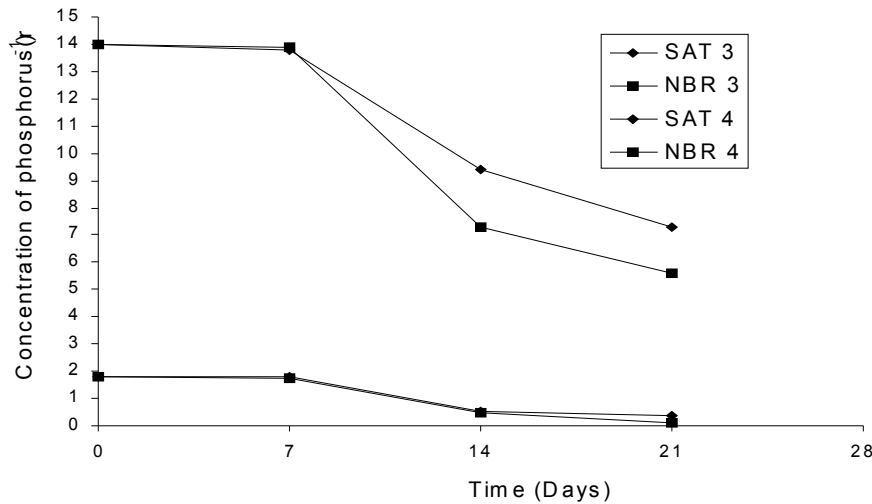


Fig. 4. Depletion of orthophosphate P from nutrient media during growth of duckweed using static aeration (SAT) and normal batch reactor (NBR) techniques.

The initial P concentration of 1.79 mg P l⁻¹ observed in both SAT 3 and NBR 3 was found to have reduced to 0.39 mg P l⁻¹ in SAT 3 and 0.12 mg P l⁻¹ in NBR 3, respectively, at the end of the experiment. P loss from the control SAT 4 and NBR 4 was found to be high, declining from an initial level of 14 mg P l⁻¹ in both cases to 7.8 mg P l⁻¹ and 5.6 mg P l⁻¹ in SAT 4 and NBR 4, respectively. Generally a lag phase was observed within the first 7 days of the experiment in all the treatments. However, a relatively high P loss followed the lag phase in all treatments. According to Iqbal (1999), the P uptake by plants growing on effluents depends largely on growth rate, harvesting frequency and availability of orthophosphate, the favoured P, for duckweed growth. Total nitrogen and orthophosphate-P content in the duckweed after 21 days is summarized in Table 4. Total nitrogen content in duckweed grown in SAT and NBR were significantly higher than nitrogen content in duckweed grown in SAT 3 and NBR 3.

TABLE 4
Total nitrogen and orthophosphate P contents in duckweed after 21 days of growth

Treatment (gkg ⁻¹ dry weight)	Total nitrogen (gkg ⁻¹ dry weight)	Orthophosphate-P
SAT3	30.38a	0.43a
SAT 4	59.11b	2.05b
NBR 3	30.10a	2.52b
NBR 4	61.32b	0.46a

Figures in columns followed by different letters are significantly different at 5% level.

Total nitrogen content of duckweed obtained in the study ranged from 30.1 to 61.32 mg N kg⁻¹ dry weight (Table 4). These values compared favourably with the values reported by both Körner & Vermaat (1998) and Vermaat & Hanif (1998). Orthophos-phate-P values measured in the duckweed species used for this study varied from between 0.43 to 2.52 mg kg⁻¹ dry weight (Table 4) were lower than reported values of Körner & Vermaat (1998) and Vermaat & Hanif (1998).

Conclusion

The study demonstrated that duckweed was capable of growing on anaerobic digester effluent provided the total ammonium nitrogen (TAN) concentration did not exceed 50 mg N l⁻¹ at pH 8. The high levels of ammonium-nitrogen, as was observed in the pure effluent and lower effluent dilutions (1/10 and 1/25), could cause growth inhibition and, consequently, death of the plants within a week of planting. Relative growth rate as well as N and P uptake from the digester effluent was substantially high, suggesting that duckweed was effective in N and P recovery from anaerobic digester effluent. Algae invasion was found to drastically reduce the yield of duckweed. This observation implied there would be the need to include algal control measures in subsequent experiments.

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References

- Alaerts G. J., Rahman Md. M. and Kelderman P.** (1996). Performance and analysis of full-scale duckweed-covered sewerage lagoon. *Wat. Res.* **30**(4): 843–852.
- APHA** (1992). *Standard Methods for the Examination of Water and Wastewater*. (A. E. Greenberg, L. S. Clesceri and A. D. Eaton, ed.). American Public Health Association, Washington DC., USA.
- Clement B. and Merlin G.** (1995). The contribution of ammonia and alkalinity to landfill leachate toxicity to duckweed. *Sci. Total Environ.* **170**: 71–79.
- Edwards P.** (1990). An alternative excreta-reuse strategy for aquaculture: The production of high-protein animal feed. In *Seminar on Wastewater Reclamation and Reuse for Aquaculture, Calcutta, India, 6–9. December 1990*.
- Gijzen H. J., and Khonder M.** (1997). *An overview of the ecology, physiology, cultivation and applications of duckweed*. Inception Report. Annex 1. Literature review. Duckweed Research Project (DWRP), Dhaka, Bangladesh. 53 pp.
- Hillman W. S., and Culley D. D.** (1978). The uses of duckweed. *Am. Sci.* **66**: 442–451.
- Hunt R.** (1978). *Plant Growth Analysis. Studies in Biology* 96. Edward Arnold, London.
- Ingermasson B., Oscarsson P., Ugglas M. A. and Larson C. M.** (1987). Nitrogen utilization in Lemna: Short term effects of ammonium on nitrate uptake and nitrate reduction. *Pl. Physiol.* **85**: 856–867.
- Iqbal S.** (1999). *Practical report*. PRISM Shobuj Shona Center, Mirzapur, Bangladesh.
- Koles S. M., Petrell R. J. and Bagnall L. O.** (1987). Duckweed culture for reduction of ammonia, phosphorus and suspended solids from algal-rich water. In *Aquatic plants for water treatment and resource recovery*. (K.R. Reddy and W. H. Smith, ed.), pp. 796–774. Magnolia, Orlando, FL.
- Körner S. and Vermaat J. E.** (1998). The relative importance of *Lemna gibba* L. bacteria and algae for the nitrogen and phosphorus removal in duckweed-covered domestic waste water. *Water Res.* **32**: 3651–3661.
- Landolt E. and Kandeler R.** (1987). The family of Lemnaceae. A monographic study: Phytochemistry, physiology, application, and bibliography. In *Biosystematic Investigations in the Family of Duckweeds (Lemnaceae)*. Veröffentlichungen des Geobotanischen Institute der ETH, Stiftung Ruelbel, Zuerich. **4**(95): 638.
- Litav M. and Lehrer Y.** (1978). The effect of ammonium in water on *Pontamogeton lucens*. *Aquat. Bot.* **5**: 127–138.
- Lüönd A.** (1983). *Growth of duckweeds (Lemnaceae) depending on nutrient supply, especially phosphorus and nitrogen*. Stiftung Ruelbel 80. Veröffentlichungen des Geobotanischen Institute der ETH Zürich.
- Monselise B. E. and Kost D.** (1993). Different ammonium uptake, metabolism and detoxification efficiencies of two Lemnaceae. *Planta* **189**: 167–173.
- Oron G.** (1994). Duckweed culture for wastewater renovation and biomass production. *Agric. Wat. Man.* **26**: 27–40
- Oron G., Porath D. and Wildschut L. H.** (1986). Waste water treatment and renovation by different duckweed species. *J. Environ. Eng.* (reaston, VA) **112**: 247–263.
- Oron G., Wildschut L. R. and Porath D.** (1984). Wastewater recycling by duckweed for protein production and effluent renovation. *Wat. Sci. Technol.* **17**: 803–817.

- Porath D. and Pollock J.** (1982). Ammonia stripping by duckweed and its feasibility in circulating aquaculture. *Aquat. Bot.* **13**: 125–131.
- Royal Tropical Institute** (1984). *Analytical Methods of the Laboratory for Soil, Plant and Water Analysis, Part II. Foliar Analysis*. Amsterdam, The Netherlands.
- Six W. and De Baere L.** (1992). Dry anaerobic conversion of municipal solid waste by means of the DRANCO process. *Wat. Sci. Technol.* **25**(70): 295–300.
- Skillicorn P., Journey W. K. and Spira W.** (1993). *Duckweed Aquaculture. A new aquatic farming system for developing countries*. The World Bank, Agriculture Division. Washington, DC. 68 pp.
- Teisseire H. and Vermaat G.** (1999). Is the 'Diuron effect' due to herbicide strengthening of antioxidative defenses of minor lemna? *Pest. Biochem. Physiol.* **66**: 153–160.
- Van der Steen P., Brenner A., Buuren J. and Oron G.** (1999). Post-treatment of UASB reactor effluent in an integrated duckweed and stabilization pond system. *Wat. Res.* **33**(3): 615–620.
- Vandenabeele J. Verhaegen K., Sudradjat R., Avnimelech Y., Van Cleemput O. and Verstraete W.** (1990). Interference in simple N-determination methods. I. Reference compounds. *Envir. Technol.* **11**: 859–862.
- Veenstra S. F. Al-Nozaily and Alaerts G. J.** (1995). Purple non sulphur bacteria and their influence on waste water stabilization pond performance in the Yemen republic. *Wat. Sci. Technol.* **31**: 141–149.
- Vermaat J. E. and Hanif K. M.** (1998). Performance of common duckweed species (*Lemnaceae*) and the western *azolla filuloides* on different types of wastewater. *Wat. Res.* **32**: 2569–2576.