

Oxygen Transport through Selected Aquatic Macrophytes

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ABSTRACT

The extent of O_2 transport from aerial plant tissue into the root zone was evaluated for several floating and emergent aquatic macrophytes that have characteristics favorable for wastewater treatment. The highest O_2 transport rates from aerial tissue into the root zone were associated with plants having a small root mass. As root mass increased, the rate of O_2 transport decreased for aquatic macrophytes evaluated. Pennywort (*Hydrocotyle umbellata* L.) had the highest O_2 transport rate of all aquatic macrophytes with an overall rate of $3.49 \text{ g } O_2 \text{ kg}^{-1} \text{ dry root mass h}^{-1}$. Pickerelweed (*Pontederia cordata* L.) had the highest O_2 transport capacity of emergent plants with a rate of $1.54 \text{ g } O_2 \text{ kg}^{-1} \text{ h}^{-1}$. Waterhyacinth [*Eichhornia crassipes* (Mart.) Solms], an important floating aquatic plant in wastewater treatment, had a transport rate of $1.24 \text{ g } O_2 \text{ kg}^{-1} \text{ h}^{-1}$. Nitrification in a waterhyacinth-based water treatment system due to O_2 transport was calculated to vary from 6 to 22 $\text{kg ha}^{-1} \text{ d}^{-1}$.

Additional Index Words: Emergent aquatic plants, Floating aquatic plants, Wastewater treatment, Nitrification, BOD, reduction.

The use of aquatic macrophytes for wastewater renovation has become an important consideration for communities with limited financial resources (Duffer, 1982;

Reddy and Smith, 1987). Pollutant removal in an aquatic plant-based wastewater treatment system is attributed to plant assimilation and biochemical or physical processes in the root zone, water column, and underlying sediment (Reddy, 1984; Good and Patrick, 1987). Both plant assimilation of pollutants and microbially mediated processes are influenced by the capacity of aquatic plants to transport O_2 into the root zone.

An anatomical adaptation of aquatic macrophytes is the development of aerenchyma cell structure, which facilitates the exchange of O_2 from aerial tissue into the root zone (Dacey, 1980). Oxygen transport through plant tissue has been established for several aquatic macrophytes (Coult and Vallance, 1958; Armstrong, 1964; Teal and Kanwisher, 1966; Armstrong, 1967). The O_2 is used for root respiration and prevents the root zone from becoming anoxic. If the amount of O_2 transported from aerial tissue into the root zone exceeds the plant demands, diffusion may occur into the surrounding aqueous media. The O_2 can then be consumed by aerobic bacteria and result in decomposition of organic matter or nitrification. Both of these transformations are critical for wastewater renovation.

The objective of this study was to determine the extent of O_2 transport from aerial plant tissue into the root zone of selected aquatic macrophytes that have characteristics favorable for wastewater treatment.

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MATERIALS AND METHODS

The apparatus used to measure O_2 transport consisted of an O_2 electrode (Yellow Springs Instrument Co., Yellow Springs, OH) connected to a PVC column sealed at one end to a PVC plate (Fig. 1). The electrode was held in place with silicon glue. The columns were 10 to 30 cm in length with a 5.1 or 7.6 cm i.d. Four columns were built for each column length and diameter combination for replication. The columns were of different length and diameter to accommodate differences in root morphology.

Two barriers were used to restrict O_2 exchange between atmosphere and water. Each barrier was evaluated for its effectiveness in preventing O_2 leakage from atmosphere into the water column. The procedure to evaluate O_2 leakage through each barrier was similar to the following procedures described for a barrier with plant.

Oxygen transport was calculated from the net change in dissolved O_2 concentration with time. Results were expressed as $g\ O_2\ kg^{-1}$ dry root mass. Oxygen transport of plants were corrected for O_2 leakage through each barrier with no plant. Aquatic plants used in this study were collected from the St. Johns River marsh. They were cultured in nutrient medium containing $NH_4-N = 10.5\ mg\ L^{-1}$; $NO_3-N = 10.5\ mg\ L^{-1}$; $PO_4-P = 3.1\ mg\ L^{-1}$; $K = 23.0\ mg\ L^{-1}$; $Ca = 20.0\ mg\ L^{-1}$; $Mg = 5.0\ mg\ L^{-1}$; $Fe = 0.6\ mg\ L^{-1}$; and micronutrients. Micronutrients were applied through liquid fertilizer (Nutrispray-Sunniland, Chase and Co., Sanford, FL) to obtain concentrations of $4\ mg\ Fe\ L^{-1}$; $0.2\ mg\ Cu\ L^{-1}$; $1.5\ mg\ Mn\ L^{-1}$; $0.04\ mg\ B\ L^{-1}$; $0.02\ mg\ Mo\ L^{-1}$; and $3\ mg\ S\ L^{-1}$.

Paraffin Oil Barrier

Water in the PVC column was pruged with N_2 gas until the dissolved O_2 concentrations were less than $0.3\ mg\ O_2\ L^{-1}$. A waterhyacinth [*Eichhornia crassipes* (Mart.) Solms] plant was placed in each container and the magnetic stirrer was turned on. Nitrogen gas was bubbled through the water column for an additional 5 min. At the end of 5 min, the aquarium stone was removed from the water column and a 1-cm layer of heavy paraffin oil was poured around the plant. The plant roots were kept below the paraffin oil layer. The dissolved O_2 concentration was recorded immediately and represented time 0 and the magnetic stirrer was turned off. At 30-min intervals, the dissolved O_2 concentration was recorded after a 30-s stir. At the end of 120 min, the paraffin oil was discarded and the plant was washed thoroughly with tap water. The relationship between O_2 transport and time was linear for 120 min and steady-state conditions were reached at 180 or 210 min.

Split-rubber Stopper Barrier

A waterhyacinth plant was placed in a no. 11 or no. 14 split-rubber stopper having a center hole of 1.7 to 2.0 cm in diam. The excess space surrounding the plant was plugged with glazing seal (used for setting glass in greenhouses). Water in the PVC columns was purged with N_2 gas for several minutes until the dissolved O_2 concentration was less than $0.3\ mg\ O_2\ L^{-1}$. A waterhyacinth plant/rubber stopper was positioned on top of the PVC column so that the roots were submerged in the water. The magnetic stirrer was turned on and N gas was bubbled for an additional 5 min. The aquarium stone was removed from the water column and the split-rubber stopper was forced into the column. The outside connection between the split-rubber stopper and the PVC column was sealed with glazing seal. The dissolved O_2 concentration was recorded immediately and represented time 0 and the magnetic stirrer was turned off. The dissolved O_2 concentration was measured at 30-min intervals after a 30-s stir. At the end of 120 min, the plant was removed from the split-rubber stopper and washed thoroughly.

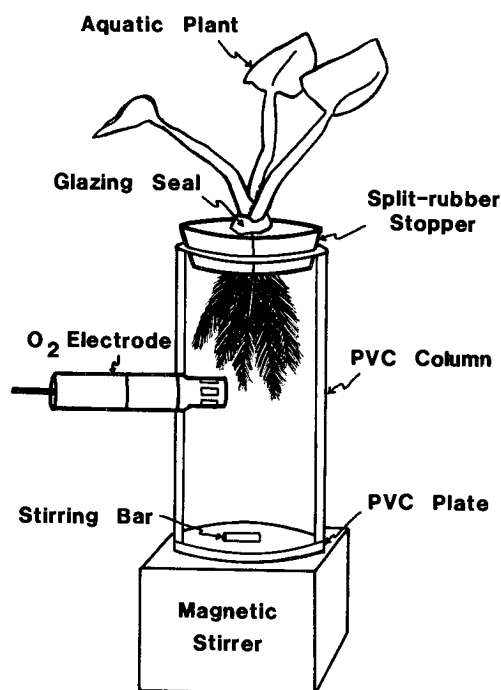


Fig. 1. Apparatus used to measure O_2 transport by aquatic macrophytes. The split-rubber stopper barrier is illustrated here.

In one study, 30 waterhyacinth plants of relatively uniform size (average root length = 6 cm and average total fresh wt. plant⁻¹ = 11 g) were grown in tubs (0.25-m² surface area and 20-cm depth) containing nutrient medium described earlier. Four plants were removed at 1, 3, 7, 10, and 13 d, and O_2 transport rates were measured using the split-rubber stopper method.

Plant Parameters

More than 30 waterhyacinth plants were evaluated for each barrier described above. Each plant was divided into shoots and roots. The length of each shoot and primary root was recorded. The leaf width and length were recorded to calculate total leaf area. The shoots and roots were oven-dried at 70°C for dry-wt. determinations.

Other Selected Macrophytes

The split-rubber stopper barrier was chosen for additional studies of O_2 transport through floating and emergent aquatic macrophytes. Floating plants included pennywort (*Hydrocotyle umbellata* L.) and water lettuce (*Pistia stratiotes* L.). Emergent plants included pickerelweed (*Pontederia cordata* L.) and cattail (*Typha latifolia* L.). The plants were divided into shoots and roots for dry weight determinations after removing them from the split-rubber stopper.

RESULTS

The leakage of O_2 from atmosphere into the water column was less for the split-rubber stopper barrier with no plant compared to the paraffin oil barrier with no plant (Fig. 2). Both barriers were effective in restricting O_2 exchange from atmosphere into the water column.

Paraffin Oil Barrier

Oxygen transport by waterhyacinth was measured for plants having a wide range of morphological differences (Table 1). The initial hypothesis was that O_2 transport

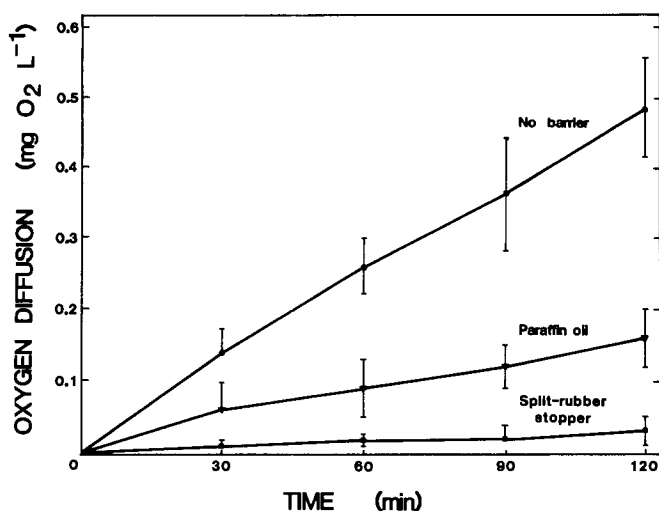


Fig. 2. Oxygen leakage through the two barriers and open water (no barrier). Vertical bars represent standard deviation.

would be related to plant morphological characteristics such as root or shoot mass, total root or shoot length, total leaf area, or shoot/root (S/R) dry weight ratio. Grouping all 34 plants together and regressing O_2 transport rates against these plant parameters indicated no significant relationships with correlation coefficients generally negative and less than $r = -0.30$. The highest correlation came between root mass and O_2 transport ($r = -0.467$). The plants were then divided into five groups based on their root mass.

Total O_2 transport ($g O_2 kg^{-1}$ dry root mass) of waterhyacinth, at 30-min intervals, is shown for both barriers in Table 2. The transport values were corrected for O_2 leakage through each barrier. There was considerable variation in O_2 transport regardless of root mass category or barrier.

A summary of O_2 transport rates ($g O_2 kg^{-1}$ dry root mass h^{-1}) of waterhyacinths are shown in Table 3. They were corrected for O_2 leakage through each barrier. The highest O_2 transport rates from aerial plant tissue into the rhizosphere were associated with plants having a small root mass. Plants having a root mass < 0.10 g had O_2 transport rates of $3.73 g O_2 kg^{-1} h^{-1}$ compared to $0.11 g O_2 kg h^{-1}$ for plants having a root mass > 1.00 g. Root systems increase in size and weight as a waterhyacinth plant develops, suggesting a physiological aging difference for O_2 transport. Older plants and plants with extensive rooting systems probably consume more of the transported O_2 due to higher respiration rates.

Table 1. Morphological characteristics of waterhyacinth plants used in O_2 transport studies.

Plant parameter	Paraffin oil		Split-rubber stopper	
	Mean \pm SD†	Range	Mean \pm SD	Range
Root mass, g	0.65 ± 0.63	0.07-2.97	0.22 ± 0.19	0.03-0.69
Shoot mass, g	1.49 ± 0.76	0.29-3.40	0.97 ± 0.41	0.25-2.18
S/R	3.65 ± 2.26	0.74-8.71	8.33 ± 7.63	1.77-33.7
Root length, cm	613 ± 285	116-1235	459 ± 211	180-1004
Shoot length, cm	79 ± 21	31-115	65 ± 13	35-95
Leaf area, cm^2	141 ± 55	34-269	120 ± 42	30-206

† SD = standard deviation.

Table 2. Total O_2 transport with time for waterhyacinth with two barriers. Values represent means and standard deviations.

Root mass plant ⁻¹	Time, min				
	30	60	90	120	n†
g	g $O_2 kg^{-1}$ dry root mass				
	Paraffin oil barrier				
< 0.10	3.06 ± 0.33	4.48 ± 0.27	5.85 ± 0.21	7.45 ± 0.17	2
0.10-0.25	1.06 ± 0.56	2.09 ± 0.68	3.00 ± 0.82	3.76 ± 1.10	8
0.26-0.50	0.37 ± 0.34	0.54 ± 0.37	0.77 ± 0.39	1.01 ± 0.43	8
0.51-0.99	0.21 ± 0.12	0.24 ± 0.15	0.31 ± 0.23	0.37 ± 0.26	8
> 1.00	0.09 ± 0.07	0.11 ± 0.10	0.20 ± 0.19	0.21 ± 0.18	8
	Split-rubber stopper barrier				
< 0.10	1.06 ± 0.78	2.27 ± 1.24	3.71 ± 1.89	4.78 ± 2.35	10
0.10-0.25	0.96 ± 0.69	1.52 ± 0.90	2.07 ± 1.09	2.55 ± 1.22	10
0.26-0.50	0.18 ± 0.15	0.33 ± 0.15	0.52 ± 0.23	0.61 ± 0.22	8
0.51-0.99	0.09 ± 0.08	0.18 ± 0.18	0.20 ± 0.24	0.23 ± 0.29	4

† n = number of plants.

Anatomical differences may also exist between younger and older plants.

Split-rubber Stopper Barrier

Results obtained by this method were similar to those observed for the paraffin oil barrier method. Oxygen transport rates were highest for waterhyacinths having a small root mass (Table 3). The O_2 transport rate was $2.39 g O_2 kg^{-1} h^{-1}$ for plants having a root mass < 0.10 g compared to $0.12 g O_2 kg^{-1} h^{-1}$ for plants with a root mass > 0.50 g.

An additional experiment was conducted to examine plant age on O_2 transport. Developing plants, with similar root lengths and masses, were clipped from waterhyacinth plants and cultured in nutrient medium. The plants at Day 1 had dry root masses < 0.04 g. At 13 d, the plant dry root mass ranged from 0.21 to 0.51 g. Oxygen transport rates decreased rapidly from Day 1 to Day 7 and continued to decrease throughout the 13 d of incubation (Fig. 3), indicating the effect of increasing root mass on decreasing O_2 transport.

Other Selected Macrophytes

A similar trend of decreasing O_2 transport with increasing root mass was shown for the other floating and emergent aquatic macrophytes evaluated (Table 4). Pennywort had the highest O_2 transport rate of all aquatic macrophytes evaluated with an overall rate of $3.49 g O_2 kg^{-1} h^{-1}$. Pennywort has a much smaller and finer root

Table 3. Summary of O_2 transport rates of waterhyacinth using two barriers. Values represent means and standard deviations.

Root mass	Paraffin oil barrier	n	Split-rubber stopper barrier	n†
	g $O_2 kg^{-1} h^{-1}$		g $O_2 kg^{-1} h^{-1}$	
< 0.10	3.73 ± 0.09	2	2.39 ± 1.18	10
0.10-0.25	1.88 ± 0.55	8	1.27 ± 0.61	10
0.26-0.50	0.50 ± 0.22	8	0.31 ± 0.11	8
0.51-0.99	0.19 ± 0.14	8	0.12 ± 0.14	4
> 1.00	0.11 ± 0.09	8		
Avg	0.91 ± 1.06		1.24 ± 1.15	

† n = number of plants.

Table 4. Oxygen transport rates of floating and emergent aquatic macrophytes. Transport values represent means and standard deviations.

Plant	Root mass plant ⁻¹	O ₂ transport	n†
	g	g O ₂ kg ⁻¹ h ⁻¹	
	<u>Floating aquatic macrophytes</u>		
Pennywort	0.02-0.05	3.95 ± 1.86	18
	0.06-0.12	2.49 ± 1.05	8
	0.02-0.12	3.49 ± 1.77	26
Waterlettuce	0.05-0.25	0.30 ± 0.13	10
	<u>Emergent aquatic macrophytes</u>		
Pickerelweed	0.03-0.06	1.72 ± 0.87	15
	0.07-0.14	0.61 ± 0.22	3
	0.03-0.14	1.59 ± 0.90	18
Cattail	0.02-0.10	1.39 ± 1.49	4
	0.11-0.53	0.19 ± 0.15	14
	0.02-0.53	0.45 ± 0.92	18

† n = number of plants.

system compared to waterhyacinth and can tolerate colder ambient air temperatures. The low rate of O₂ transport by water lettuce was surprising since the root structure of this plant is similar to that of waterhyacinth.

The O₂ transport rates for floating and emergent aquatic macrophytes are within the range of O₂ transport rates from submerged vascular macrophyte roots to oxygen-depleted water in light reported by Sand-Jensen et al. (1982). Sand-Jensen et al. (1982) determined that O₂ rates are faster in light than in darkness for submerged macrophytes. However, light does not appear to influence the rate of O₂ transport into roots of terrestrial plants (Armstrong, 1967). The rapid O₂ exchange between aerial plant tissue and atmosphere via stomates should limit the role of photosynthesis in O₂ transport of floating and emergent aquatic macrophytes.

DISCUSSION

An oil or wax layer has been used extensively to restrict O₂ diffusion in O₂ transport studies (Coult and Vallence, 1958; Armstrong, 1964, 1967). Paraffin oil contains a high percentage of O₂ (Armstrong, 1967) and further use of the oil is difficult due to mixing and contamination with water. The effect of paraffin oil on plant cell wall permeability and on physiological processes is unknown. Armstrong (1964, 1967) coated the region of plants exposed to a layer of paraffin oil with lanolin to prevent oil from penetrating into the plant. Disposal problems associated with paraffin oil suggested a different approach to study O₂ transport. The split-rubber stopper barrier was developed as an alternative method to paraffin oil in these studies. The glazing seal used with the split-rubber stopper barrier should not effect cell wall permeability if the plants are handled carefully. The glazing seal can be recycled indefinitely compared to the difficult separation of paraffin oil and water.

The waterhyacinth plants used in this study came from several sources and had a wide range of morphological characteristics (Table 1). The average root mass was 0.65 g for plants used with the paraffin oil barrier compared

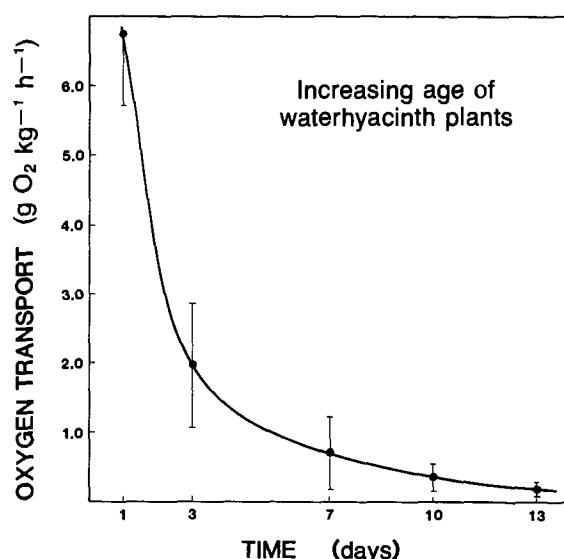


Fig. 3. Effect of increasing age (increasing root mass) of waterhyacinth plants on O₂ transport rates. Vertical bars represent standard deviation.

to 0.22 g for plants used with the split-rubber stopper barrier. The smaller root mass in the split-rubber stopper experiments also reflects the higher average of S/R dry weight ratio and lower total root length for these plants. Although no significant relationships could be established between plant morphological characteristics and O₂ transport, the decreasing O₂ transport with increasing root mass provides indirect evidence for a physiological aging effect on O₂ release from the root system.

Oxygen transport rates were always less for the split-rubber stopper barrier when comparing root mass categories (Table 3). The O₂ transport for all plants combined was 0.91 g O₂ kg⁻¹ h⁻¹ for the paraffin oil barrier and 1.24 g O₂ kg⁻¹ h⁻¹ for the split-rubber stopper barrier. The higher transport rate for the split-rubber stopper barrier was due to evaluating more plants having a root mass of 0.02 to 0.25 g.

Assuming an overall O₂ transport rate of 1.0 g O₂ kg⁻¹ h⁻¹, O₂ release with time into the root zone can be calculated if plant density and S/R dry weight ratio are known. Once released, the O₂ can be utilized during microbial respiration, nitrification or other processes requiring O₂. Operational plant density (to achieve maximum yield) of waterhyacinth was found to be in a range of 500 to 2000 g dry wt. m⁻² (Reddy and DeBusk, 1984). The S/R dry weight ratio increases with increasing nutrient availability. Plants cultured in primary sewage effluent typically have a S/R dry weight ratio of 4.0 or higher (Reddy, 1984). A density of 500 to 2000 g m⁻² and a S/R dry weight ratio of 4.0 gives a root mass of 100 to 400 g m⁻². Therefore, 100 to 400 mg of O₂ m⁻² h⁻¹ is released from the root tissue into the surrounding aqueous medium.

The stoichiometric nitrification equation requires 4.3 mg O₂ for every milligram of NH₄-N nitrified (Barnes and Bliss, 1983). If O₂ consumption is due entirely to nitrification, 23 to 92 mg of NH₄-N will be converted to NO₃-N m⁻² h⁻¹ or 6 to 22 kg ha⁻¹ d⁻¹. Hauser (1984) calculated

a theoretical nitrification rate of $8 \text{ kg ha}^{-1} \text{ d}^{-1}$ due to O_2 transport by waterhyacinths in ponds receiving secondary effluent. High nitrification rates are possible when waterhyacinths are used as final filteres to polish secondarily treated sewage effluent. These effluents typically have low BOD_5 concentrations. Potentially, these rates will be about three times higher if pennywort is used in the system as compared to waterhyacinth. Oxygen transport rate of pennywort was $3.49 \text{ g O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ as compared to $1.24 \text{ g O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ by waterhyacinth. However, operation plant density of pennywort is only 250 to 650 g m^{-2} (Reddy and DeBusk, 1984) and the S/R dry weight ratio is much higher than waterhyacinth. Therefore, the O_2 released into the root zone may be similar to waterhyacinth per unit area.

Wastewaters vary considerably in the amount of inorganic N concentrations. Generally primary sewage effluent contains appreciable amounts of $\text{NH}_4\text{-N}$ but minimal $\text{NO}_3\text{-N}$. However, primary sewage effluent also contains higher levels of BOD_5 , which consumes O_2 . Oxygen consumption is, therefore, directly related to the efficiency and populations of the microbial flora for converting BOD_5 to CO_2 or $\text{NH}_4\text{-N}$ to $\text{NO}_3\text{-N}$. Preliminary results (data not shown) indicated that O_2 transport from aerial plant tissue into the root zone supports BOD_5 reduction in primary sewage effluent.

Wastewater treatment with waterhyacinth plants has resulted in a reduction of 90% of BOD_5 (McDonald and Wolverton, 1980; Reddy et al., 1985). The stoichiometric equation of glucose oxidation to CO_2 requires $1.1 \text{ mg O}_2 \text{ mg}^{-1}$ glucose oxidized. If O_2 consumption was due entirely to glucose oxidation, 91 to 364 mg of glucose would be converted to $\text{CO}_2 \text{ m}^{-2} \text{ h}^{-1}$ or 22 to 87 $\text{kg ha}^{-1} \text{ d}^{-1}$. These removal rates may be substantially increased by using plants with high O_2 transport capability, such as pennywort. Overall reduction of BOD_5 is influenced by sedimentation and filtration by plant roots in addition to oxidation (Reddy et al., 1985).

Harvesting should enhance O_2 transport in waterhyacinth-based water treatment systems as plants reproduce vegetatively in response to opened areas. The young offsets will release more O_2 from the less extensive rooting system during the early stages of plant development. Harvesting should enhance BOD_5 reduction and nitrification in waterhyacinth-based wastewater treatment system. However, harvesting increased plant assimilation of N (DeBusk et al., 1983; Hauser, 1984). Therefore, the increased nitrification may not be noticed as the $\text{NO}_3\text{-N}$ is rapidly removed by plant assimilation. When plant density is near maximum, plant uptake of N is assumed to be minimal and nitrification/denitrification is thought to be the major pathway of N loss in waterhyacinth systems (DeBusk et al., 1983; Weber and Tchobanoglous, 1986).

The general trend of decreasing O_2 transport with increasing root mass suggests that O_2 transport by aquatic macrophytes will display seasonal fluctuations based on the active growing season for each plant. A greater O_2 transport rate should be expected as young plants develop and BOD_5 reduction or nitrification in wastewater will

be maximum during the active growing season for each plant.

There was wide variability in O_2 transport for the aquatic plants evaluated in this study. The two barriers were effective in preventing O_2 leakage and the advantages or disadvantages of each barrier were described. The O_2 transport rates were not highly correlated to plant morphological characteristics but root mass appeared to influence exchange of O_2 from the plant to water. As the root mass increased, the O_2 transport rate decreased. The effects of O_2 transport on nitrification or BOD_5 reduction in wastewaters needs further investigation.

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