

## ARTÍCULO DE REVISIÓN

## Algunos aspectos interesantes de sistemas de humedales a escala de laboratorio y de banco en México

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### Resumen

Los países con economías emergentes como México, tanto desde el punto de vista económico como tecnológico encaran problemas de disposición de aguas residuales no tratadas o pobremente tratadas. Los humedales artificiales (HA) o construidos (HC) pueden representar una opción para sus comunidades rurales ya que integran procesos físicos, químicos y biológicos logrando la remoción de contaminantes presentes en las aguas residuales, pudiéndose utilizar una vez tratadas para uso agrícola o industrial. Se realizaron experimentos con reactores a escala de laboratorio y de banco para evaluar el papel que juegan las plantas acuáticas (*Phragmites australis*, *Typha latifolia*, *Thalia geniculata*) en la remoción de contaminantes medidos como demanda química de oxígeno soluble ( $DQO_{soluble}$ ), conductividad eléctrica (EC), nitrógeno (N) y evaluando también potenciales de oxidación-reducción o redox ( $E_h$ ),  $RA_{1,2}$ , empleando controles sin macrofitas,  $RA_{a,b}$ , todos empacados con escoria volcánica (i.e., *tezontle* en Náhuatl, la lengua azteca) con capas de diferentes granulometrías. Los reactores fueron iluminados durante 16 horas por día empleando lámparas de tubos fluorescentes con características similares a la luz natural. Ambos, los sistemas con y sin plantas se mantuvieron inundados para eliminar el ingreso de aire por convección. Se utilizó agua residual sintética preparada diariamente disolviendo sacarosa,  $(NH_4)_2SO_4$  y  $Na_3PO_4$  con una  $DQO_{soluble}$  experimental promedio de 450 mg/L. Los tiempos de residencia hidráulica (TRH) de los reactores de laboratorio fueron de 0.5 y 1 días. Los reactores a escala de banco tenían valores de TRH de 14.7 y 21 d (tasas de flujo de 10 y 7L d<sup>-1</sup>). Se tomaron muestras de agua residual a la salida de cada reactor y a las profundidades de 2, 10 y 30 cm respecto al nivel del agua de los reactores para poder observar el perfil de remoción del nitrógeno (nitrógeno total Kjeldahl, NTK), la  $DQO_{soluble}$ , la CE y los potenciales redox,  $pE_h$ , empleando metodología estandarizada para la determinación de la  $DQO_{soluble}$  y nitrógeno y para las medición de la CE y los  $pE_h$  se emplearon electrodos comerciales. Las comunidades microbianas que proliferaban en la zona radicular fueron recolectadas al terminar los experimentos. Los métodos para su evaluación fueron: (a) Preparaciones frescas, (b) Tinción diferencial y Gram y (c) Inoculaciones en medios selectivos. Se usaron para la parte (c) dos técnicas, aislamiento por cuadrantes y extensión superficial, usando diferentes cantidades de inóculo (una muestra microbiológica con un alambre de platino para el aislamiento por cuadrantes y un volumen de muestra fijo de 0.1 mL por placa para la extensión superficial. Los cultivos fueron incubados por 24 horas a 37°C. Los resultados obtenidos indican que los reactores ( $RA_{1,2}$ ) mostraron los valores más altos para la remoción de NTK (90 y 80%), 54% para  $DQO_{soluble}$  y 9.3% para la CE y los valores de  $pE_h$  presentaron un comportamiento oscilante a 10 cm de profundidad (en la zona radicular) para los reactores con planta de-

+ This paper is based on some previous research communications that have appeared in several publications: Behavior of redox potentials in artificial wetlands models: A tool for controlling its efficiency. Alejandro Guido-Zárate, Germán Buitrón, Petia Mijaylova-Nacheva, Carmen Durán-de-Bazúa. En Communicating current research and educational topics and trends in applied microbiology. Ed. A. Méndez-Vilas. Formatex. Series ISBN-13:978-84-611-9421-6. Vol. 2. ISBN-13: 978-84-611-9423-0. Badajoz, España. Pp. 594-601 (2007); Artificial wetlands performance: nitrogen removal. Carmen Durán-de-Bazúa, Alejandro Guido-Zárate, Talía Huanosta, Rosa Martha Padrón-López, Jesús Rodríguez-Monroy. Water Science & Technology-WST. 58(7):1357-1360 (2008); Pollutants removal in a lab-scale constructed wetlands model system / Remoción de contaminantes en un sistema modelo de humedales artificiales a escala de laboratorio. Alejandro Guido-Zárate, Carmen Durán-de-Bazúa. Technol. Ciencia Ed. (IMIQ). 23(1):15-22 (2008); Efecto de la presencia de hidrofitas (*Typha latifolia*) sobre los potenciales redox (reducción-oxidación) en columnas empacadas a escala de laboratorio / Effect of hydrophytes (*Typha latifolia*) presence on the redox potentials (reduction-oxidation) in lab scale packed columns. María Guadalupe Soto-Esquivel, Sandra Guzmán-Aguirre, Adriana G. Mejía-Chávez, Rolando S. García-Gómez, Carmen Durán-de-Bazúa. Technol. Ciencia Ed. (IMIQ). 24(1):5-11 (2009)

mostrando el efecto fotosintético en la iluminación y oscuridad, respectivamente, comparados con los reactores control ( $RA_{a,b}$ ) sin planta (38.1% para  $DQO_{soluble}$  y 4.4% para CE). El análisis de los resultados indica que estas diferencias fueron estadísticamente significativas ( $P < 0.05$ ). Los valores de  $DQO_{soluble}$  y CE disminuyen a medida que la profundidad es mayor siendo mayor la remoción de ambos parámetros a la profundidad de 10 cm para el reactor con planta resaltando la importancia de la zona radicular de la planta en la eficiencia de remoción del sistema. Las comunidades microbianas con bacterias Gram (-) fueron más abundantes en la zona radicular comparadas con los reactores control a escala de laboratorio.

**Palabras clave:** Remoción de contaminantes, nitrógeno total Kjeldahl, demanda química de oxígeno, conductividad eléctrica, potenciales redox, comunidades microbianas en la zona radicular, humedales artificiales, humedales construidos.

## Some behavioral aspects of laboratory and bench scale artificial wetlands model systems in Mexico

### Abstract

Artificial or constructed wetlands (AW or CW)) may become a feasible option for the water problems faced by emerging economies countries, such as Mexico, both from the technical and economical point of view, because they are integrated wastewater treatment systems. In them, through physical, chemical, and biological processes, pollutants can be efficiently removed from wastewaters especially in rural areas allowing its use for agricultural and/or industrial purposes. Experiments have been performed at lab and bench scales using reactors to evaluate the role played by plants (*Phragmites australis*, *Typha latifolia*, *Thalia geniculata*) in pollutants removal measured as soluble chemical oxygen demand ( $COD_{soluble}$ ), electrical conductivity (EC), nitrogen (N), and evaluating redox potentials ( $E_h$ ) too. Laboratory and bench scale reactors were built using plastic containers, 25 and 147 liters, respectively, planted with aquatic macrophytes,  $RA_{1,2}$  and its controls without macrophytes,  $RA_{a,b}$ , all packed with volcanic slag (i.e., *tezontle* in Aztec language) at different granulometry layers. The reactors were illuminated during 16 h a day using fluorescent tube lamps with natural light characteristics. Reactors were kept flooded to minimize the effect of convective oxygen transfer through air sucking. Synthetic wastewater was daily prepared by dissolving sucrose,  $(NH_4)_2SO_4$ , and  $Na_3PO_4$  rendering an approximate concentration of 450 mg  $COD_{soluble}$  /L. Hydraulic residence times (HRT) for lab reactors were 0.5 and 1 d whereas for bench scale systems were 14.7 and 21 d (flow rates of 10 and 7 L d<sup>-1</sup>). Samples of wastewaters at the inlet and outlet as well at 2, 10, and 30 cm depth from the water level were taken, and the N (total Kjeldahl nitrogen, TKN),  $COD_{soluble}$ , EC, and  $pE_h$  were determined using the standard methodology and commercial electrodes, respectively. Microbial communities proliferating in the root zone of lab reactors were assessed using (a) Fresh preparations, (b) Differential Tinctions and Gram, and (c) Inoculations in selective media. For part (c) two techniques were applied, isolation by quadrants and surface extension, using different inoculum quantities (platin wire for quadrants isolation and a fixed sample volumes of 0.1 mL per plate for the surface extension incubating during 24 hours at 37°C. Results obtained indicate that plants have a marked beneficial role ( $RA_{1,2}$ ) with removal rates of 90 and 80% as TKN, 54 y 9.3%, for  $COD_{soluble}$  and EC, and the  $pE_h$  values presented an oscillation behavior at 10 cm depth (root zone) for planted reactors demonstrating the photosynthetic effect with illumination and darkness, respectively, compared with the control reactors. The analysis of the data indicated that these differences were statistically significant ( $P < 0.05$ ). Values for  $COD_{soluble}$  and EC diminish as depth increases having the highest removal at 10 cm where the plant root system is located indicating the importance of the plant presence for the pollutants removal phenomena. Microbial communities with Gram (-) bacteria were more abundant in the root zone when compared with the control lab reactors.

**Keywords:** Pollutants removal, total Kjeldahl nitrogen, chemical oxygen demand, electric conductivity, redox potentials, root zone microbial communities, artificial wetlands, constructed wetlands.

## 1. Introduction

Constructed or artificial wetlands are based on the following fundamental principles: biochemical activity of microorganisms, the oxygen supply leaked from root plants, and a bed material who serves as a support for both microorganisms and plants, this material may operate as a filter for many particles [1-2] and ion exchange matrix as well. These elements eliminate dissolved and suspended substances in the wastewater and biodegrade the organic compounds towards its mineralization. Constructed or artificial wetlands can be employed as a complementary system in the existent treatment plant [3] to improve water quality (polishing), and can also be used as the main treatment systems for small communities [4-5].

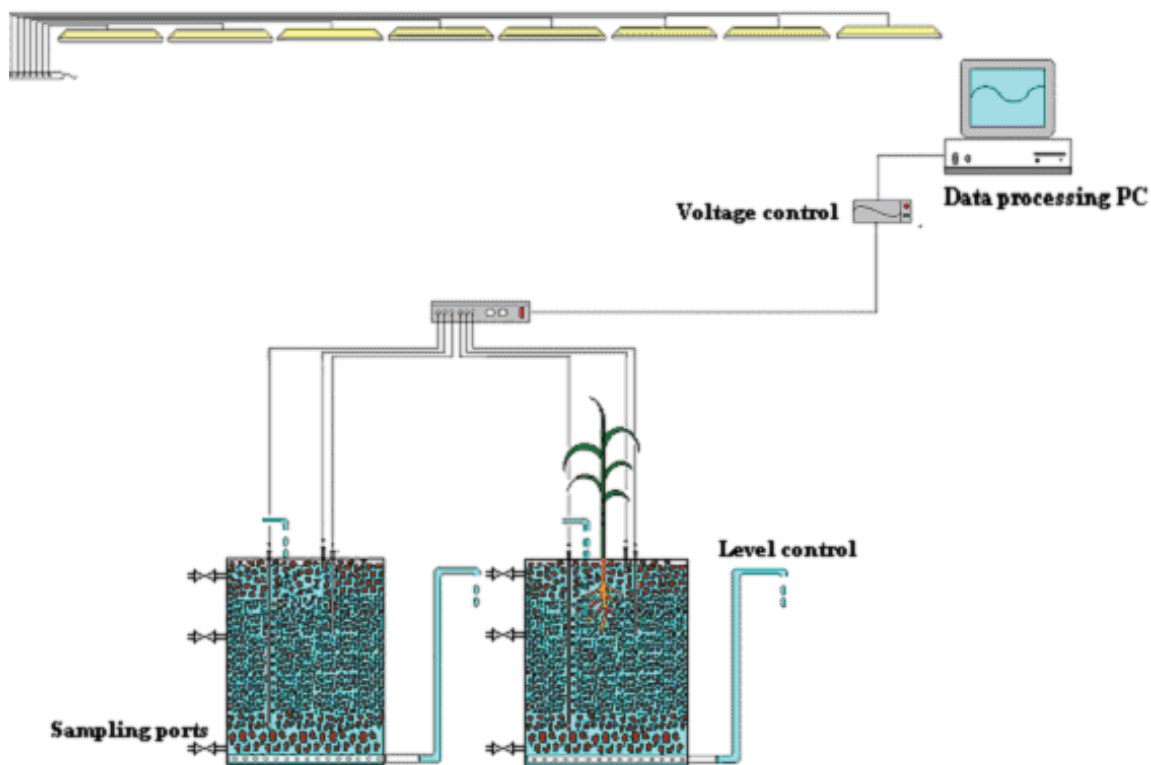
Thus, the objectives of this research were to evaluate

the performance of these systems with emphasis on the role of the hydrophytes at laboratory and bench scale using as variables soluble chemical oxygen demand, electrical conductivity removal efficiencies, reduction-oxidation potentials, and assessing the microbial communities growing on the root zone. A highlight was to follow the behavior during the night when no photosynthesis is carried out by these hydrophytes.

## 2. Materials and methods

### Laboratory-scale reactors

Laboratory-scale reactors were constructed. The reactors consisted of cylindrical plastic containers, 30 cm diameter and 35 cm height (Fig.1).



**Fig. 1a.** Lab scale Experimental system (with a duplicate for each reactor) (taken from Guido-Zárate et al. [6, 14])

Three sampling ports were placed at 2, 10 and 30 cm depth (from the water level) to take a representative sample from each point. Water level was controlled by using a latex hose set at 2 cm below the packing material level. The reactors were filled completely with volca-

nic slag (*tezontle*, in Aztec language, meaning stone as light as hair) arranged in layers with different granulometric sizes to have a suitable hydraulic flow [7]. One shoot of plant (*Phragmites australis*) was placed centrally inside the reactors ( $RA_{1,2}$ ). The second set of reactors

remained unplanted as a control ( $RA_{a,b}$ ). Electrodes to measure redox potentials were installed at three different heights from the upper columns surface down (electrode A at 2cm, electrode B at 10cm, and electrode C at 30cm). Calibration of the electrodes is presented in the literature [8]. Reactors were set to have flooding conditions to minimize the effect of convective oxygen transfer through air sucking [9]. Hydraulic residence times (TRH) studied were 1 and 2 days.

Synthetic wastewater was used for both reactors, and was daily prepared by dissolving sucrose,  $(NH_4)_2SO_4$ , and  $Na_3PO_4 \cdot 12H_2O$  in a 40L container in tap water, resulting in a C:N:P ratio of 15:1:0.1, and a  $COD_{soluble}$  of 450  $mgO_2/L$  which is a typical value for domestic effluents or sewage [10]. Daily inflow for each reactor was settled so that hydraulic residence times considering porosity factor (51%) were 0.5 and 1 days [11]. Reactors were placed in a controlled room illuminated with fluorescent tubes lamps (day light lamps) using a timer for 16hr on and 8 hrs off. After reactors reached steady state (4 weeks approximately, plant height = 53 cm), soluble chemical oxygen demand and electrical conductivity were measured, in addition, samples of wastewater were taken at 2, 10 and 30 and tested for  $COD_{soluble}$  and EC to insight in each reactor. Test for  $COD_{soluble}$  was performed by using standard methodology [12], corrected previously by Oaxaca-Grande [13]. Electrical conductivity was measured by using a multi parameter portable tester. Water was fed centrally to minimize diffusion effects through the reactors. Level control was possible by using a latex hose set at 2 cm below packing material level as mentioned above. Relative humidity, lab temperature was recorded to observe possible drastic change in these parameters during the experiment.

### Bench-scale reactors

The system located in Mexico City, comprised five reactors with dimensions of 0.51 m long x 0.34 m width x 0.85 m deep (Figure 2), two filled with gravel and three with volcanic slag at different granulometry layers [7] made of plastic boxes (147-L); four reactors were originally planted with common reeds (*Phragmites australis*) and cattails (*Typha latifolia*) with an average plant density of 6 plants  $m^{-2}$ ; these were operated in a vertical mode and under ambient conditions. The theoretical hydraulic residence time (HRT) was 14.7 d and the flow rate of 10  $L d^{-1}$ . Values for dissolved oxygen (DO), pH, temperature, total Kjeldahl nitrogen (TKN), chemical oxygen demand the influent and effluent were determined [12]. The water

level was maintained 2 cm below the surface with a PVC pipe. Data used for this research were taken from a period comprised in the spring-summertime (seven months) from April to October 2005 with temperatures at noon around 25°C (although in late evening to early hours come down to 8-12°C). The second system located in Tabasco, comprised five reactors with the same dimensions like the system located in Mexico City, and in this case, four reactors where planted with mixed hydrophytes: cattails (*Typha latifolia*) and *Thalia eniculata*, a tropical aquatic plant. For this system, the equivalent period evaluated (seven months) was from July 2004 to February 2005 (socalled "dry season" or fall-winter). The flow rate was 7  $L d^{-1}$  and the HRT of 21 d. The average ambient temperature during the experiment was 25°C, the climate in the location of the experiment is predominantly tropical with annual highest average precipitations of 339 mm [14]. Both systems had a control reactor filled with the same material and operated in the same fashion. Values for dissolved oxygen (DO), pH, temperature, total Kjeldahl nitrogen (TKN), and chemical oxygen demand in the influent and effluent were determined using the same methods as for the system located in Mexico City. For both systems, synthetic water was prepared by dissolving sugar,  $C_{12}H_{22}O_{11}$ , ammonium sulphate,  $(NH_4)_2SO_4$ , and potassium phosphate,  $H_2KPO_4$ , the resulting COD,  $N-NH_4^+$  concentrations were 380 and 22.5  $mg L^{-1}$  respectively for both systems. The systems were operated in a vertical mode and the intermittently feed. The COD and TKN loading rates in each case were 146  $kg ha^{-1} d^{-1}$  and 0.03-0.6  $kg TKN ha^{-1} d^{-1}$ , respectively.

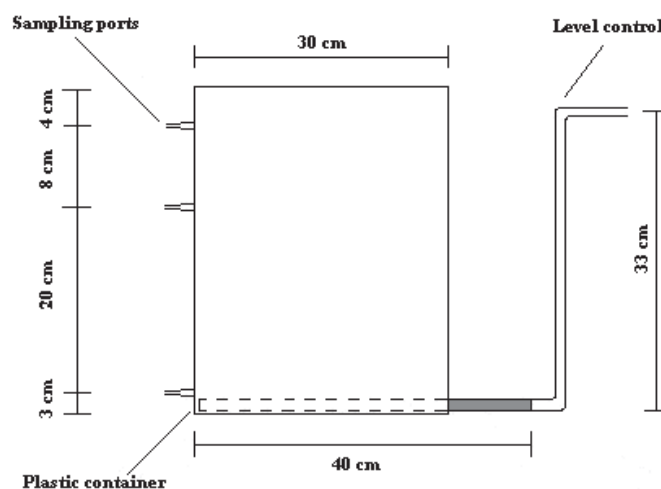


Fig. 1b. Lab reactors dimensions (taken from Guido-Zárate et al. [6, 15])

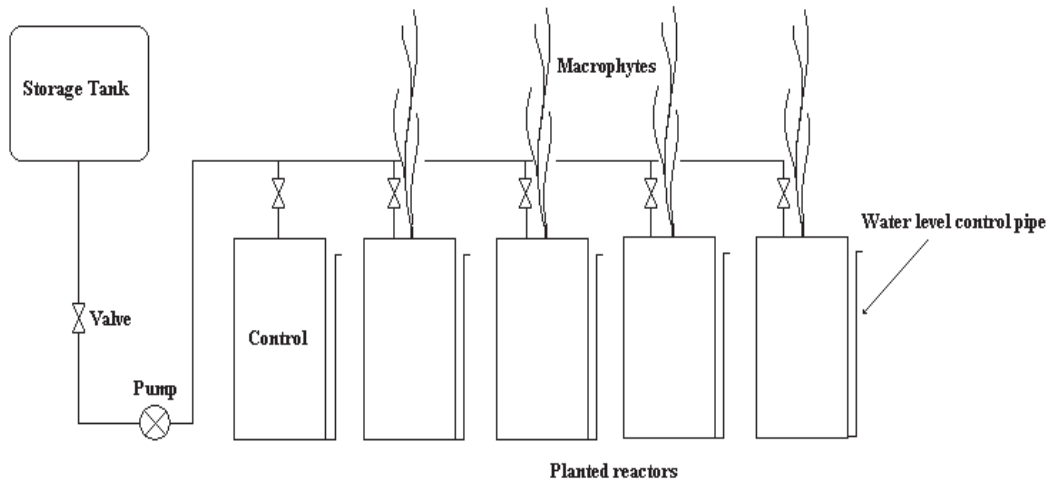


Figure 2a. Experimental bench scale systems (taken from Durán-de-Bazúa et al. [15])

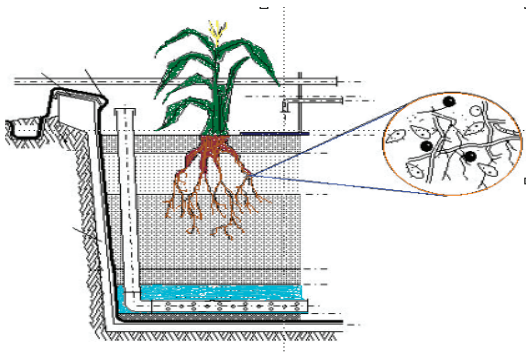
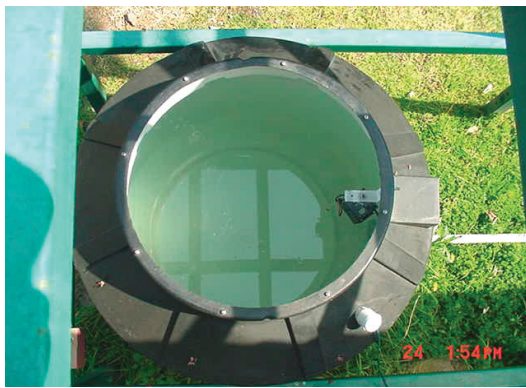


Figure 2b. System in Mexico City, Mexico [16]



Popal camalote  
 (*Thalia geniculata*)



Cattails (*Typha latifolia*)



Figure 2b. System in Tabasco, Mexico [17]

### 3. Results and discussion

#### Lab scale reactors

Each lamp irradiated an average of  $1.38 \text{ Wm}^{-2}$ , giving a total of  $20.6 \text{ Wm}^{-2}$ , equivalent to a summer morning in Mexico City [18], constantly maintained along the experiments [19].

#### Chemical oxygen demand values ( $\text{COD}_{\text{soluble}}$ )

Table 1 and Figs. 3 to 6 show the average percentages of  $\text{COD}_{\text{soluble}}$  removals. These results clearly show a reduction of this parameter in the planted reactor ( $\text{RA}_1$ ) and its control ( $\text{RA}_2$ ). The water samples were taken at the outlet of each reactor during the steady state for both illumination and darkness periods. For illumination period it can be seen that reactor  $\text{RA}_1$  (planted) presented the highest  $\text{COD}_{\text{soluble}}$  removal (effluent  $\text{COD}_{\text{soluble}}$  of  $216.2 \text{ mg O}_2/\text{L}$ ), indicating that plant has an important effect in the reduction of  $\text{COD}_{\text{soluble}}$ . This effect lays on the fact that during illumination period, organic matter oxidation is enhanced due to the gas transfer modified by the irradiation status and the oxygen supplied to the reducing surroundings, creating a very thin oxygen layer trough which aerobic bacteria can satisfy their oxygen demand. The root zone can serve as a support material for heterotrophic bacteria as well [1] which consume the oxygen supplied by plants (photosynthetic and through air-spaces found in plants roots), increasing degradation as mentioned above (Table 1).

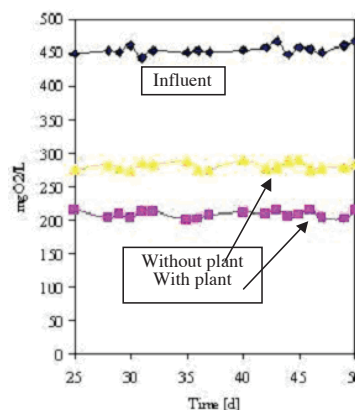
**Table 1.** Average  $\text{COD}_{\text{soluble}}$  removal percentage (steady state) during illumination and darkness periods\*

Reactors	Illumination	Darkness
$\text{RA}_{1,2}$ (planted)	54.1	43.1
$\text{RA}_{a,b}$ (unplanted control)	39.8	38.7

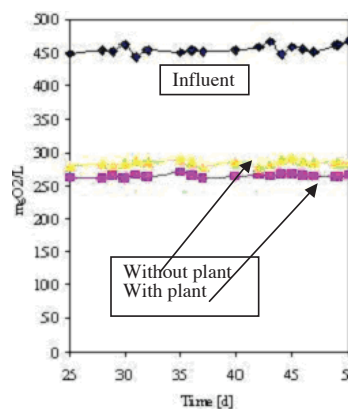
\*Each value represents the mean for the steady period

Anoxic bacteria can also degrade organic material via "anaerobic respiration" where the ultimate electron acceptor is not molecular oxygen but oxygen attached to sulfates and nitrates mainly, conditions in both reactors prevailed anaerobic and  $\text{H}_2\text{S}$  was confirmed by simply smelling the water. It can be surmised that in the bulk volume, sulfate reduction is taking place and organic oxidation is carried out by the mechanism mentioned above. The  $\text{COD}_{\text{soluble}}$  removal for control reactor  $\text{RA}_2$  (unplanted) was lower in comparison with  $\text{RA}_1$  (effluent

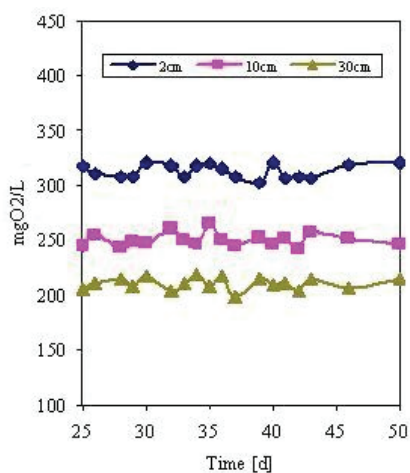
value of  $281.4 \text{ mgO}_2/\text{L}$ ) due to the unplanted status. It can be seen that  $\text{COD}_{\text{soluble}}$  values at the effluent of each reactor during dark periods are higher than those for illumination period supporting the belief that plants oxygen supply increases with irradiation status. A small variation in reactors  $\text{RA}_2$  was observed, because the oxygen transfer from atmosphere is consumed by heterotrophic bacteria in the surface water layer diminishing  $\text{COD}_{\text{soluble}}$ . Chemical oxygen demand value at the outlet of reactor  $\text{RA}_2$  was  $284 \text{ mg O}_2/\text{L}$ .  $\text{COD}_{\text{soluble}}$  profiles for both illumination and darkness periods indicate that  $\text{COD}_{\text{soluble}}$  diminishes as depth increases for both reactors. It is interesting to notice that for planted reactor, the  $\text{COD}_{\text{soluble}}$  removal is higher at 10 cm (root zone) in comparison to unplanted reactor. Stratification of  $\text{COD}_{\text{soluble}}$  removal percentage was found in both reactors for both periods, however no difference in this percentage when considering the illumination status for unplanted reactor was found.



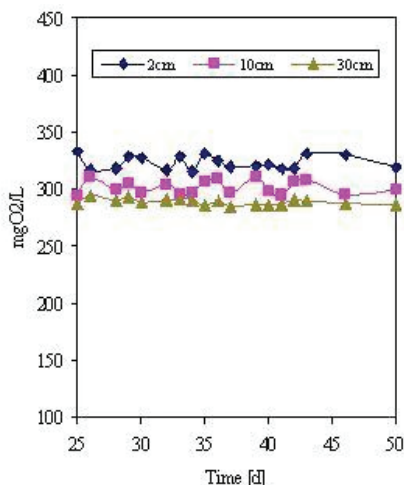
**Fig. 3.**  $\text{COD}_{\text{soluble}}$  effluent variations at steady state during illumination period (Influent,  $\text{RA}_{1,2}$  with plant,  $\text{RA}_{a,b}$  no plant) (Taken from Guido-Zárate et al. [6, 15])



**Fig. 4.**  $\text{COD}_{\text{soluble}}$  effluent variations at steady state during darkness period ( $\text{RA}_1$  with plant,  $\text{RA}_{1,2}$  with plant,  $\text{RA}_{a,b}$  no plant) (Taken from Guido-Zárate et al. [6, 15])

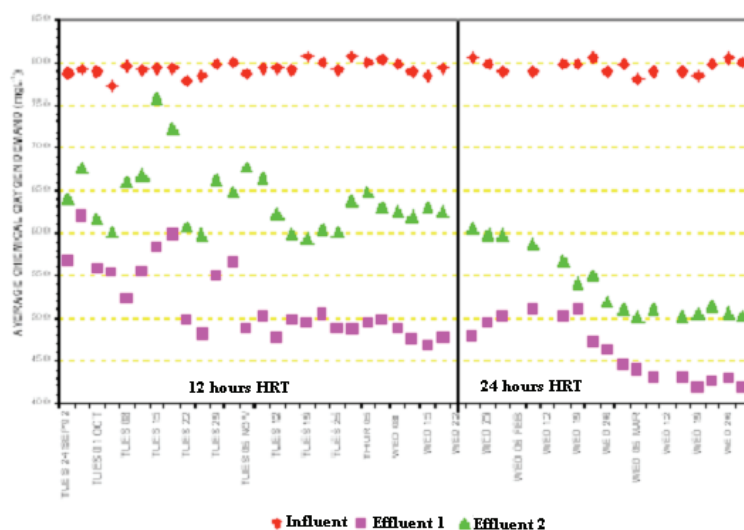


**Fig. 5.**  $COD_{soluble}$  profile ( $RA_{1,2}$  with plant) during illumination period (Taken from Guido-Zárte et al. [6, 15])



**Fig. 6.**  $COD_{soluble}$  profile ( $RA_{a,b}$  no plant) during illumination period (Taken from Guido-Zárte et al. [6, 15])

The analysis of the results indicated that differences for the  $COD_{soluble}$  profiles were statistically significant ( $P < 0.005$ ). The hydrophyte definitely improves the degradation of organics when compared with the equivalent reactors without vegetation. Unplanted reactor can remove  $COD_{soluble}$  but only through anaerobic degradation since the flooding conditions and no gas transfer via plants greatly decreases the oxygen content in the bulk volume. Oxygen content in the inflow may be the unique oxygen source for organic matter oxidation in these two control reactors, both at 12 hours hydraulic residence time or at 24 hour hydraulic residence time. Hydraulic residence time also plays a role on degradation efficiency (Fig. 7).



**Fig. 7.** Average values for chemical oxygen demand values for influent and effluents for the reactors  $R_{1,2}$  (with hydrophytes, effluent 1) and for the control reactors  $R_{a,b}$  (control without plant, effluent 2) operating at 12 and 24 h hydraulic residence time, HRT (Taken from Soto-Esquivel et al. [20])

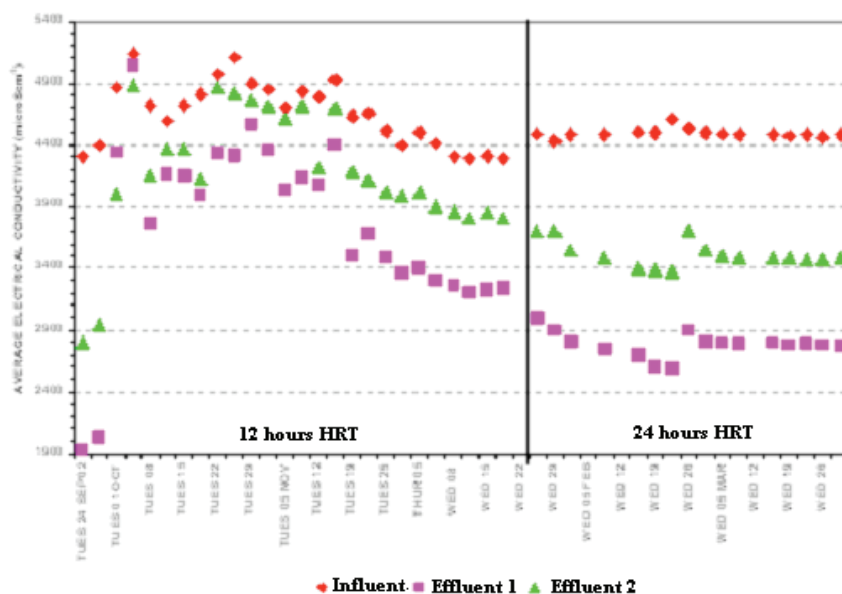
### Electrical conductivity (EC)

The variations of EC during illumination and darkness period show no significant differences between illumination and darkness period for  $RA_{1,2}$  and  $RA_{a,b}$  were observed ( $P > 0.005$ ). It is known that plants can uptake certain ions as trace nutrients and eniculate them to their tissues. Results obtained indicate that under these experimental conditions, planted reactors ( $RA_{1,2}$ ) did show a significant effect on EC removal. Ions removal through plants may not have been the main EC-removal mechanism and this may be because of the fact that plants were small (53 cm high) and that radiation characteristics did not enhance these mechanisms (temperature inside the laboratory was  $\approx 23.5^\circ\text{C}$  and relative humidity 53% during the experiment). In real constructed wetlands systems, EC removal via evapotranspiration can be an important process due to the high plant density (more than two meters high and direct solar light characteristics). EC removal percentages did not vary significantly in both illumination and darkness periods (Table 2) which indicate that illumination did not affect the ions removal mechanism under these specific experimental conditions. EC removal profiles follow the same behavior as for  $COD_{soluble}$ . Even though there EC diminishes at higher depths, the EC removal efficiency was low for both reactors. From EC removal results obtained we can conclude that although plant removed

EC by the nutrients uptake and incorporate them to their tissues, both reactors performed this task more as filters and ion exchange reactors through the support media adsorption, since under the experimental conditions, evapotranspiration-mediated CE removal was not the main factor for the reduction of EC in planted reactor. Just like for COD, hydraulic residence time (12 and 24 hours HRT) also plays a role on EC degradation efficiency (Fig. 8).

**Table 2.** Average EC removal percentage (steady state) during illumination and darkness periods (taken from Durán-de-Bazúa et al. [15])

Reactor	Illumination	Darkness
RA <sub>1,2</sub> (planted )	9.31	9.16
RA <sub>a,b</sub> (unplanted control)	4.31	4.45



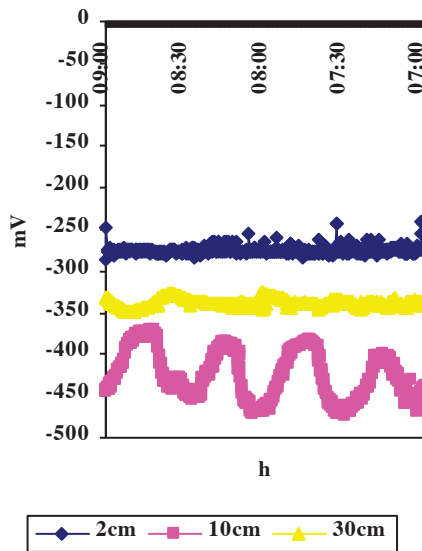
**Fig. 8.** Average values for electric conductivities of influent and effluents for the reactors R<sub>1,2</sub> (with hydrophytes, effluent 1) and for the control reactors R<sub>a,b</sub> (control without plant, effluent 2) operating at 12 and 24 h hydraulic residence time, HRT (taken from Soto-Esquivel et al. [20])

### Redox potentials, E<sub>h</sub>

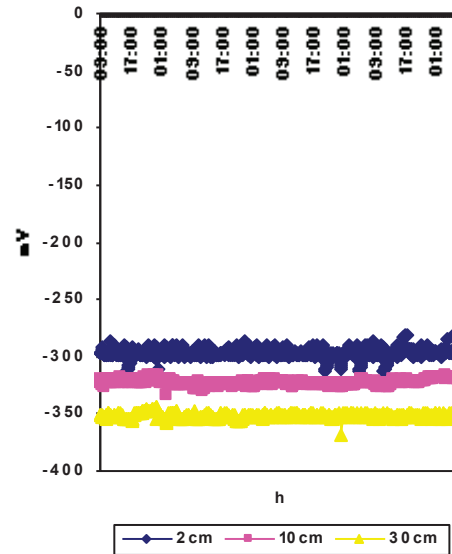
Figure 9 shows the variations of the redox potentials for reactor RA<sub>1,2</sub> (planted and open to diffusive air) during the light and darkness periods (measurements correspond to four operating days in the steady state) at the three different reactor depths (2, 10, 30 cm). It is interesting to observe the oscillating pattern of this parameter at 10 cm depth, where the root zone is. This variation demonstrates that E<sub>h</sub> is affected by the oxygen generated by the macrophyte during the illumination and darkness periods. Another interesting phenomenon observed during these experiments is that E<sub>h</sub> values are more negative than even those at 30cm deep. As this

phenomenon takes place in both reactors that contain the plant, it seems to be independent of the air oxygen that might be diffusing from the atmosphere as well as with the influent feed drops (Fig. 9). The possible explanation lays on the fact that aerobic bacteria were found in this area and they are consuming the photosynthetic oxygen for degrading the dissolved pollutants, and therefore, decreasing the E<sub>h</sub> values even more than in the 30 cm deep area. It is important to consider that, in these two reactors, the plant has a very small root zone, and although photosynthetic oxygen is generated the amount is not enough to change the redox conditions near the electrode. The two reactors without plant have a perfectly stratified pattern (Fig. 10).





**Fig. 9.** Redox potentials variations ( $E_h$ ) in reactor  $RA_{1,2}$  (with plant) during illumination and darkness periods



**Fig. 10.** Redox potentials variations ( $E_h$ ) in reactor  $RA_{a,b}$  (no plant) during illumination and darkness periods

(Taken from Guido-Zárate et al.[21])

These aerobic bacteria seem to be consuming photosynthetic oxygen as they metabolize wastewater compounds, reducing overall  $COD_{soluble}$  values, during the illumination period. This is confirmed with the darkness results, where removal is very similar to the control reactors without plant (no photosynthetic oxygen available), acting as sequencing reactors. These results suggest that oxygen entering with the influent drops undergoes a stratification (lower concentration at higher depth) and the concomitant stratification of  $E_h$ .

The pattern in  $RA_{a,b}$  remained without change during illumination and darkness periods. This behavior is similar to that reported by some authors [22], where the change of  $E_h$  at different light intensities was studied.  $E_h$  values at the surface (2 cm depth) of reactor  $RA_{a,b}$  were less negative due to the presence of the oxygen

contents at inflow wastewater drops that is transferred from atmosphere.

### Microbiological results of the root zone

Table 3 presents the results obtained from the microbiological tests carried out with the biomass collected from the root zone of the lab reactors. It is clear that the presence of photosynthetic oxygen promotes more proliferation of microorganisms. As control liquids, tap water and recently prepared synthetic wastewater were also tested. Although there are organisms its presence is equivalent to the control lab reactors. In Table 4 are shown some of the results for the liquid effluent samples taken at 2 and 10 cm depth. It is interesting to corroborate that also the liquid carried more organisms (measured as colonies formation units, CFU) near the root zone.

**Table 3.** Results obtained from the microbiological tests of the reactors  $RA_{1,2}$  (with *Typha*) and  $RA_{a,b}$  (without plant) operating at a hydraulic residence time of 24 h

Samples	Fresh preparations	Gram tinction	Microbiological development in nutritive agar	
			Quadrant isolation	Surface extension
$RA_{1,2}$	Considerable amounts of protozoa (different sizes and high mobility)	Short Bacillus Isolations G(-)	+++	+++++
$RA_{a,b}$	Protozoa (different sizes and high mobility)	Short Bacillus Isolations G(-), Streptobacillus	++	+++++
Tap water	No microbial growth observed	No microbial presence observed	---	++
Synthetic wastewater: Sucrose, $NH_4NO_3$ , $Na_3PO_4$	No microbial presence observed	Short Bacillus Isolations G(-)	++	++++

+++++ Considerable amounts of microorganisms, + Poor amounts of microorganisms, --- No microorganisms

**Table 4.** Number of colonies forming units per milliliter, CFU mL<sup>-1</sup>, of heterotrophic bacteria (microaerobic and facultative bacteria) in the reactors effluent samples (steady state) (Factor 10<sup>4</sup>) (taken from Guido-Zárate et al. [21])

Reactor	2 cm	10 cm
RA <sub>1</sub> (planted, uncovered, open to diffusive oxygen)	2.95	4.70
RA <sub>2</sub> (unplanted control, uncovered, open to diffusive oxygen)	1.50	1.20

## Bench scale reactors

### Nitrogen removal

The systems under study performed considerable well in terms of TKN. The removal efficiencies were 90 and 80% (Mexico City and Tabasco, respectively). It is assumed in this specific case that TKN is mainly N-NH<sub>4</sub><sup>+</sup> since tap water was used to prepare the influent synthetic water and no other organic compound was added. The vertical operation mode in both systems and the intermittently feeding favoured the oxygen transfer to the system, values of the dissolved oxygen concentration in the effluent concentration were low (Table 5).

**Table 5.** Average values for dissolved oxygen (DO), pH, and temperature (°C) during the seven months of operation in the effluent for the planted reactors (taken from Durán-de-Bazúa et al., 2008) [14]

System	DO (mg/L)		PH		Temperature (°C)	
	In	Out	In	Out	In	Out
Mexico City	1.42	0.55	6.19	6.3	19	20
Tabasco	1.3	1.2	6.35	6.8	23.8	25

According to Bogdanowicz [23] and Brix [24], plants can introduce oxygen to the root zone via the lacunae system creating favorable conditions close to the plant roots for nitrifying bacteria and for the mineralization of nutrients (e.g. N-NH<sub>4</sub><sup>+</sup>). The removal efficiencies for these parameter agree with those reported by Kantawanichkul and Somprasert [25]. The removal of organic matter was higher in the Mexico City system than that of the Tabasco system. This was as expected since the slightly lower temperatures were believed to promote the growth and better adaptation of the macrophytes (Table 5), as well as the eniculate ion of molecular oxygen. It is important to mention that the plant density of the Mexico City system increased consider-

ably at the end of the experiment compared with the Tabasco system. The values of COD removal percentage for the Tabasco system was about 35 and 53 for the Mexico City, due perhaps to the longer HRT in the Tabasco case and, again, the availability of dissolved molecular oxygen.

Remembering the lab scale experiments, influent pH values were maintained between 7 and 8 whereas the effluents for both systems at the two HRT studied were for the reactor RA<sub>1,2</sub> between 5.5 and 6, and between 4.5 and 5.5 for the control reactors without hydrophyte RA<sub>a,b</sub>. Anaerobic or anoxic conditions are present in the lower part of the reactors, since the root zone is confined to their upper part. In the control reactors, since there is no photosynthetic oxygen, probably volatile organic acids that reduce pH values [26].

Values for the reduction-oxidation potential (E<sub>h</sub>) for the Mexico City system, were measured and they were positive during the entire experiment (40-290 mV), this suggests that the roots introduced enough oxygen to create oxidizing conditions in the root zone. These values agree with those reported by Huanosta [27] in which the E<sub>h</sub> was measured in the bench scale planted reactors (*Phragmites australis*) at different depths (2, 10, and 30 cm), the values oscillated from -45 to +180 mV.

Looking at the pH values (Table 3) for both systems, these slightly increased but the effluent pH values were still low for the volatilization to become important (pK<sub>a</sub> for NH<sub>4</sub><sup>+</sup>/NH<sub>3</sub> is 9.25 [28]). The pH values are favourable for nitrifying bacteria (i.e. pH 6-9) [29-30]. Therefore, the results suggest that nitrification and denitrification played an important role in removing nitrogen from the synthetic water.

Results from these studies confirm that nitrogen was removed slightly better at higher altitudes (in Mexico City up to 93.3% as ammonia N, and 93.1% as total nitrogen with no removal in the control reactor), than at sea level (in Tabasco up to 81% as ammonia and as total N with no removal in the control reactor), and that hydrophytes definitely have an effect on nitrogen removal. It is interesting to mention that in the laboratory system in the dynamic phase when the hydrophytes were in the first growth period (Figure 11), carried out at higher altitude (Mexico City), the changes in nitrogen, both from ammonia and from nitrates were consequent with the re-

sults with bench scale artificial wetlands with fully grown hydrophytes (Figures 12a and b) [27]). *Typha latifolia* y *Thalia eniculata* are tropical plants that can be used

with the advantage of selling its flowers as ornamental products giving an added value to the treatment systems as well as to produce art crafts with the canes.



Fig. 11. Lab-scale artificial wetland system with *Phragmites australis* (at the beginning of the experiments, left, and at the end of the experiments, right)

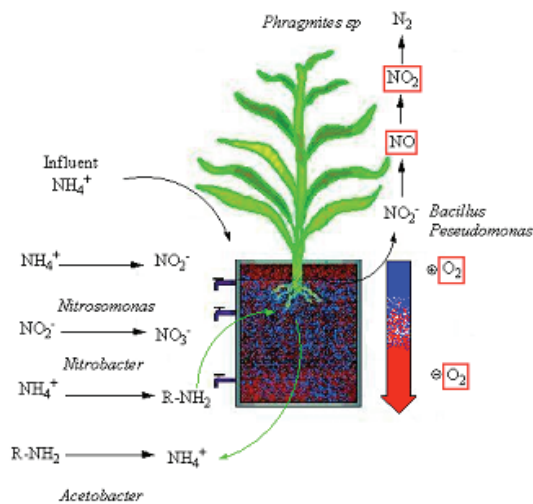


Fig. 12a. Exemplification of the nitrogen compounds fate depending upon the type of microorganisms acting in the rhizosphere: Results for N-NH<sub>4</sub><sup>+</sup>

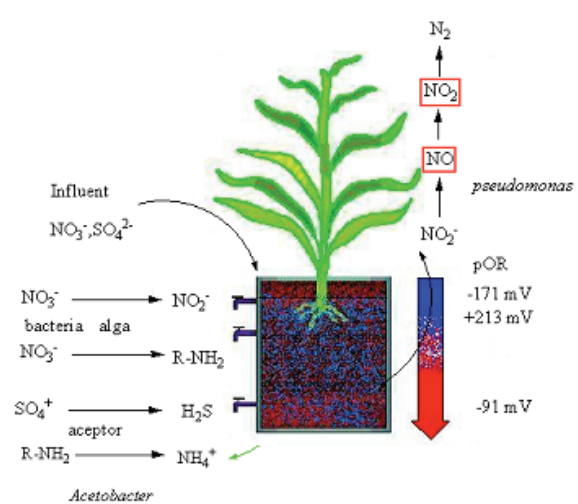


Fig. 12b. Exemplification of the nitrogen compounds fate depending upon the type of microorganisms acting in the rhizosphere: Results for N-NO<sub>3</sub><sup>-</sup>

#### 4. Conclusions

Considering the objectives of this research, the evaluation of the performance of the lab scale and bench scale systems simulating the operation of artificial wetlands using as variables soluble chemical oxygen demand, electrical conductivity removal efficiencies,

reduction-oxidation potentials, and assessing the microbial communities growing on the root zone, as a conclusion, it can be said that the oxygen supplied by the plant to the root zone of the reactor shows a greater effect in organic matter degradation than that transferred directly from atmosphere through diffusion in the control reactors without any plant.

From  $E_h$  results is possible to state that oxygen, both generated by photosynthesis as well as that transported from air through the aerenchyma [31-32], had the main effect on redox potentials variation and that under this specific experimental conditions, no important effect of atmospheric oxygen was observed.

Chemical oxygen demand removal was higher for planted reactors ( $RA_{1,2}$ ) and these values were very similar (54.1, 43.1, and 53.9, 40.9 for illumination and darkness periods, respectively) suggesting that atmospheric oxygen had not an important effect on the elimination of this parameter. Microbial organisms abundance confirm these results.

From the bench scale experiment it could be assessed that nitrogen was removed slightly better at higher altitudes (in Mexico City up to 93.3% as ammonia N, and 93.1% as total nitrogen, with no removal in the control

reactor without hydrophytes), than at sea level (in Tabasco, up to 81% as ammonia, and as total N, with no removal in the control reactor). Hydrophytes definitely have an effect on nitrogen removal. *Typha latifolia* y *Thalia geniculata* are tropical plants that can be used with the advantage of selling its flowers as ornamental products giving an added value to the treatment systems as well as to produce artcrafts with the canes.

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