TÍTULO DEL TRABAJO

"Changes on microfauna community in experimental subsurface flow constructed wetlands according to the type of organic matter supplied"

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FIGURAS Y TABLAS

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CHANGES ON MICROFAUNA COMMUNITY IN EXPERIMENTAL SUBSURFACE FLOW CONSTRUCTED WETLANDS ACCORDING TO THE TYPE OF ORGANIC MATTER SUPPLIED

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ABSTRACT
The effect of administration of particulate and soluble organic matter to experimental subsurface flow constructed wetlands has been evaluated by mean of changes on microfauna composition. Two experimental constructed wetlands with a length of 1.0 m, a width of 0.5 m and a wetted depth of 0.3 m have been monitored for a period of three months with both physical-chemical and biological analyses carried out on weekly basis. The results obtained suggests that although there are no differences in terms of pollutants removal efficiency, the microfauna composition within the first third of each wetland is highly dependant on the source of organic matter supplied. Specifically, the first third of the wetland fed with starch is characterised by the dominance of ciliates, while in case of the first third of the wetland fed with glucose, the dominant group appear to be the heterotrophic microflagellates.
KEY WORDS: wetlands, flagellates, ciliates,

CAMBIOS EN LA COMUNIDAD DE MICROFAUNA EN HUMEDALES CONSTRUIDOS ATENDIENDO AL TIPO DE MATERIA ORGÁNICA ADMINISTRADA

RESUMEN
El efecto de la administración de materia orgánica particulada y soluble en humedales construidos de flujo subsuperficial (HCFSS) se ha evaluado mediante el estudio de las comunidades de microfauna. Para tal objetivo se han utilizado dos HCFSS de 1m de longitud por 0.5 m de ancho, con una profundidad de la lámina de agua de 0,3m. Los sistemas experimentales descritos han sido estudiados mediante ensayos físico-químicos y biológicos realizados semanalmente durante un período de 3 meses. Los resultados obtenidos sugieren que, aunque la eficiencia de eliminación de contaminantes no difiere de un sistema al otro, la composición de la microfauna en el primer tercio de los humedales es altamente dependiente del tipo de materia orgánica administrada. El primer tercio del humedal alimentado con almidón se caracteriza por una elevada presencia de protozoos ciliados, mientras que en el caso del humedal alimentado con glucosa el grupo predominante son los microflagelados heterótrofos.
PALABRAS CLAVE: Humedales construidos, flagelados, ciliados

1.-INTRODUCTION

Although the importance and the role of the microfauna community in conventional wastewater treatment processes have been extensively documented: removal of dispersed bacteria (Curds, 1982), presence of toxic compounds (Salvadó *et al*., 2001; Madoni *et al*., 1998), organic load (Salvadó & Gracia, 1993), sludge health (Madoni, 1994), effluent quality in terms of BOD_5 (Salvadó *et al*., 1995), etc.. There is a lack of information concerning the role of such microorganisms in subsurface flow constructed wetlands (SSFCW), and the scarce information available in this research field is more focused in the bacterivorous activity of ciliates (Decamp, *et al*., 1999) rather than the use of the whole microfauna community as bioindicator of plant performance and operational conditions. On the other hand, although there are several reports that evidences that the treatment kinetics of biological wastewater treatment systems can be highly dependant on the particle size distribution in the influent wastewater (Levine, 1985), there is scarce scientific information available to which extend the particulate organic matter can affect the treatment efficiency of a subsurface constructed wetland, though an excessive amount of suspended solids is considered one of the major problems of SSFCW clogging (USDA, 1995).

The aim of this paper is to evaluate, for the first time, the differences in the whole microfauna community (not only ciliated protozoa but also flagellates and small metazoa) due to the status of the organic matter (particulate and soluble) supplied to two experimental SSFCW.

2.-MATERIAL AND METHODS

The experiments were conducted in two SSF with a length of 1.0 m, a width of 0.5 m and a wetted depth of 0.3 m. Both SSF were daily fed with synthetic wastewater (see composition in table 1). In one system the source of organic matter was glucose and in the other potato starch commercialised by Merck. The two SSFCW were operated at 6 days of mean retention time and the total COD at the influent was of approximately 300 mg/L. Some of the most important physical-chemical parameters analysed according to APHA's Standard Methods (APHA, 1995) are summarised in table 2. Samples for microfauna examination were taken twice a week from near the inlet (first third of the SSFCW), at the middle (second third of the SSFCW) and near the outlet (last third of the SSFCW) for a period of three months. Furthermore, samples for microfauna examination represented a mix of interstitial liquid and gravel biofilm, and were collected within the first 5 cm of the water level. From those collected samples, protozoa (ciliates and flagellates) and small metazoa were counted by mean of 25?L sub-samples under optical microscope. Identification of ciliated protozoa was carried out "in vivo" according to Foissner *et al*., (1991, 1992, 1994) and, eventually, a special silver technique described by Fernández-Galiano (1994) was used for specific identification. Flagellates protozoa were classified according to its body size into two groups: microflagellates (<25 µm body size) and macroflagellates (>25 µm body size) and small metazoa were classified to a family level according to Koste (1978a, b).

3.-RESULTS AND DISCUSSION

Although there are not differences in terms of pollutant removal efficiency comparing both SSFCW (see table 2), which is probably due to the fact that the starch is highly biodegradable even in anaerobic circumstances (Sanders et al., 2000), the results obtained in terms of microfauna analyses, indicate that there are large differences on microfauna composition near the inlet of the system (Figure 1). Thus, flagellates smaller than 25 ?m and ciliates appear to be the dominant group at the beginning of the system fed with glucose and starch respectively. In addition, the abundance distribution of ciliates and metazoa between both SSFCW is also different along the system. As it is already described by Decamp (1999), we found that the higher the distance to the inlet of the system the lower the microfauna abundance. Despite of this, we found that the 90% of metazoa and ciliates of the SSFCW fed with glucose were equally distributed between the beginning and the middle of the system, while in case of the SSFCW fed with starch, the 90% of ciliates and metazoa was found at the beginning of the system (Figure 2). This different microfauna distribution is probably due to differences in organic matter accumulation, which in case of starch is mainly placed at the beginning of the SSFCW, while in case of glucose (that diffuses better than starch) it is supposed to go further (up to the middle of the system). On the other hand, the fact that the samples analysed in the middle and final part of the SSFCW fed with starch show microfauna populations with very low abundances in ciliates and metazoa suggests that the presence of ciliates and metazoa must be somehow linked to the presence of starch. The hypothesis suggested by the authors to explain this fact is that the presence of starch granules at the beginning of the system makes the biofilm more porous, offering an excellent interstitial microhabitat for ciliates, which are more capable of feeding on surfaces than flagellates (Figure 3). Also the analysis of ciliates at specific level revealed differences between the

SSFCW fed with glucose and starch. However, the differences at specific level within the ciliates group is not due to the presence of different species between both experimental SSFCW, but in the relative abundance of the same species (Figure 4). In this sense, it is possible to see that the greatest difference between both SSFCW concerns to the hymenostomate ciliate *Trimyema compressum*, which is an organism typically found in anaerobic environments. The reason why the abundance of this ciliate is higher in case of the system fed with glucose is not clear, but may be due to one of the following hypothesis or even a combination of both :

(i).-Glucose is more easily biodegraded than the starch and so the oxigen consumption is higher in case of the SSFCW fed with glucose.
(ii).-The size of the mouth of T. Compressum is smaller compared to the rest of the ciliates present in this study. It is obvious to think of the size of the mouth as a limiting factor for the sort of prey consumed; in this sense it is probably that in case of the SSFCW fed with glucose the amount of dispersed bacteria is higher and so the abundance of a small hymenostomate could be greater.

4.-CONCLUSIONS

-The analysis of the structure of the microfauna community in the first part of a SSFCW is different according to the sort of organic matter supplied to the system (glucose/starch). Flagellates and ciliates are the dominant microfauna groups in the SSFCW fed with glucose and starch respectively.
-The abundance distribution of ciliates and metazoa along the both experimental SSF studied is different. The 90% of the abundance of these microfauna groups is placed at the beginning of the system in case of the SSF fed with starch, while in case of the system fed with glucose, the 90% of ciliates and metazoa is equally distributed up to the middle of the experimental system. This fact evidences the great capacity of microfauna to indicate the presence of organic matter.
-The abundance of *T. Compressum* in the SSFCW fed with glucose is higher than in case of the SSF fed with starch. However, the reason why is not clear yet.

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6.-TABLES

Table 1. Composition of synthetic wastewater for 20 L of total volume.

	Glucose	**Starch**
Glucose (D+) (gr) ($C_6H_{12}O_6$)	12.0	-
Starch (gr) $(C_6H_{12}O_6)_n$	-	11.4
NH_4Cl (gr)	3.0	3.0
K_2PO_4 (gr)	0.26	0.26

Table 2. Physical-chemical parameters during the experimental period.

		Conduct. (? s/cm)	COD (mg/L)	NH^{4+} -N (mg/L)	NO_3^- -N (mg/L)
Starch	Influent	294,0 ?343,7	216,4 ?97,2	19,7 ?6,8	0,2 ?0,6
	Effluent	653,8 ?479,0	6,6 ?11,9	12,3 ? 2,5	0,4 ?0,3
Glucose	Influent	295,1 ?337,3	259,7 ?108,7	19,2 ?6,6	0,2 ?0,6
	Effluent	782,9 ?481	16,9 ?23,2	9,6 ?2,5	0,3 ?0,3

7.-FIGURES

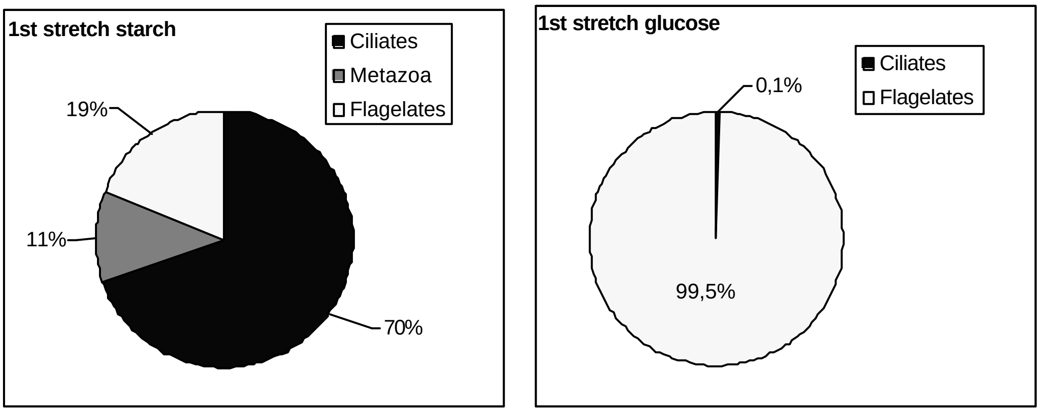

Figure 1. Percentage of abundance for the microfauna groups analysed at near the inlet of both systems. *Picture on the left*: system fed with starch; *picture on the right*: system fed with glucose.

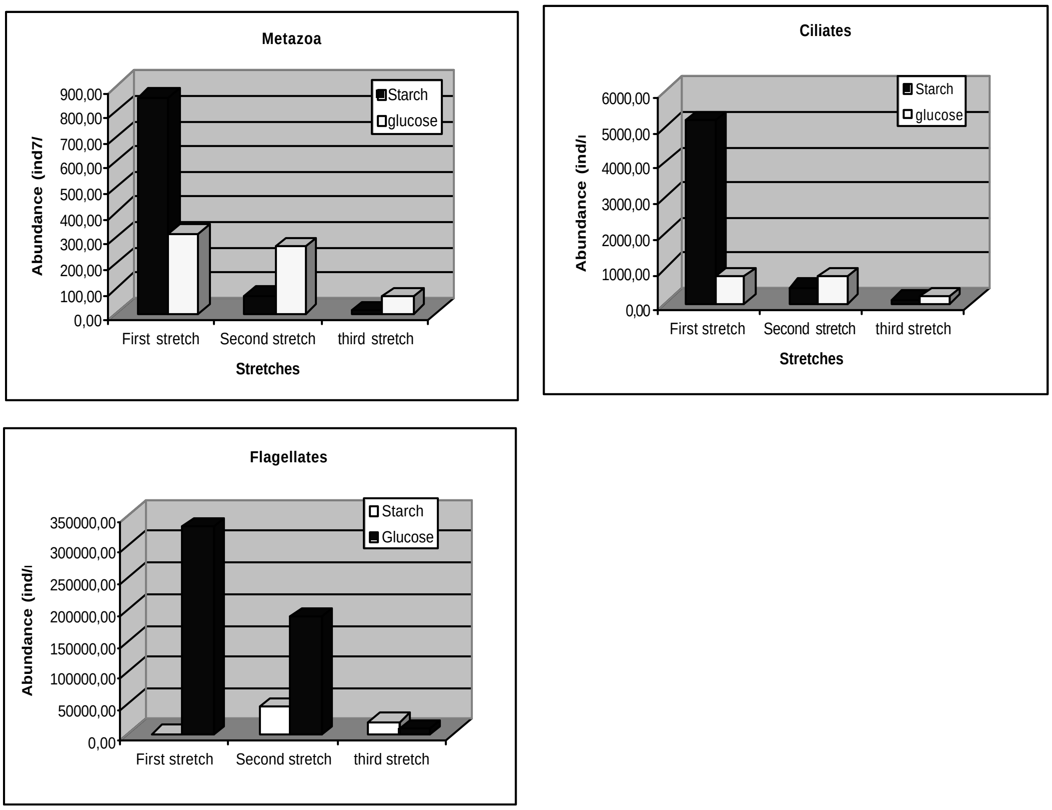

Figure 2. Comparison of Abundance of microfauna groups along both experimental SSF. *Metazoa*: upper left picture; *ciliates*: upper right picture and *flagelates*: down picture.

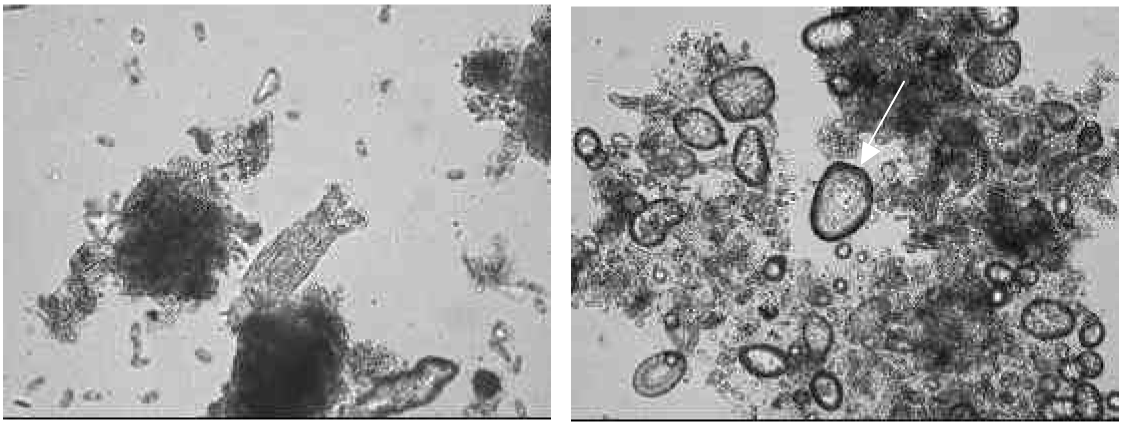

Figure 3. Microscope pictures (10x magnification) from the beginning of both experimental SSF. *Left*: SSF fed with glucose; *right*: SSF fed with starch.

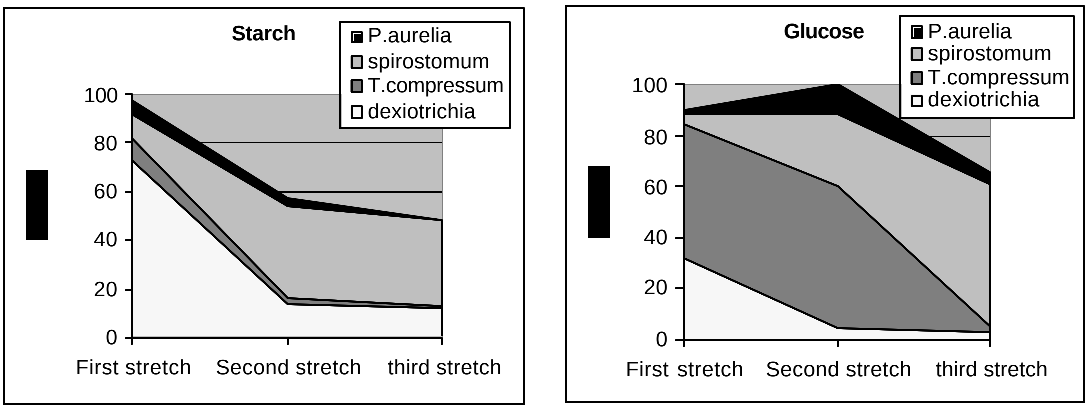

Figure 4. Percentage of abundance for the most abundant ciliates species along the experimental SSF. *Right*: SSF fed with glucose; *left*: SSF fed with starch. The white arrow indicates starch granule.