REVIVING KÄTHE SEIDEL'S LEGACY TO UNIVERSITY OF MINHO

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ABSTRACT

In the 80's Prof. Käthe Seidel was in Portugal by invitation of the University of Minho (UM). The relationship established led to the donation of Seidel's laboratory to UM, to create a Limnological Laboratory. Some institutional and personal reasons rendered this unviable and until now, Seidel's legacy at UM is resumed to some German bibliography, equipment and unpublished work.

Prof. Kickuth was Seidels' disciple. His technology is based on a rooted emergent macrophyte system of horizontal subsurface flow that relied on optimized substrate planted with *Phragmites*. Aiming to determine the efficiency of Kickuth's process and to understand the role of the biotic communities presented on these optimized substrates, mensal sampling campaigns were made in a Constructed Wetland (CW) located in the North of Portugal.

The sampling campaigns began on January 2005. Inlet (IN) and outlet (OUT) wastewater simple samples and substrate samples were taken from one of the two CW's beds. *In situ* determinations of temperature, pH and redox potential were recorded, and IN and OUT wastewater samples were analysed for BOD_5, COD and TSS; estimated densities of some microbiological communities were assessed by the recovery of colony formed units (CFU/ml) on solid culture media, using the spread technique.

The results obtained show a very good efficiency on organic load removal (values of 80-90%) accompanied by higher values in percent removal of the estimated heterotrofics CFU/ml. The total and fecal coliforms removal was also high (90-100%). The substrate's biodiversity characterized by optical microscopy allowed the identification of both protozoa and metazoa (mainly nematodes and annelids). The sampling and quantification methodologies of the biota *taxa* aren't optimized. As the knowledge of this "black box" effective function may contribute to increase the credibility of these sustainable systems, it seems pertinent to proceed with these studies.

KEYWORDS:

efficiency, Kickuth, microbiological communities, Seidel, substrate's biodiversity, wastewater.

INTRODUCTION

Although known in Portugal since 1980's, the constructed wetlands (CW) are beginning to be a choice of some municipalities to solve the problem of domestic wastewater treatment of small communities, particularly those located in rural areas.

That is the case of the "Sistema Multimunicipal de Abastecimento de Água e de Saneamento do Vale do Ave" (Multimunicipal System of Water Supply and Sanitation of the *Vale do Ave*) which include the collection, the treatment and the distribution of water to three of the the Alto Minho' municipalities and the gathering, the treatment and rejection of the wastewaters produced in the ensemble of the eight municipalities of medium and high river Ave. The choice of constructed wetland systems for treatment of the domestic wastewaters of the rural communities of the region, namely Salamonde I (Municipality of Vieira do Minho), are an example of the attention and care with the environment and the sustainable management of water and wastewater.

However, this low technology alternative faces in Portugal the unfamiliarity and incredibility of the general public and the distrust of many that only see macrophytes, namely *Phragmites*, as infesting plants. Therefore, the need to monitor this kind of wastewater treatments is increased by the need to give more credit to this economic green technology. Furthermore, there is a little number of companies with know-how in design and construction of these systems, and the effectiveness of the good performance of these systems and their divulgation depends on the cooperation and exchange of knowledge between managers and University.

Käthe Seidel's conclusions about the influence of higher plants on ballast substances, toxins and on microbiological organisms, in particular pathogenic organisms in contaminated waters (Seidel *et al*., 1978), must then be updated in order to enhance the utilization of this technology. The substrate community of constructed wetlands is made up both of the microflora (mainly bacteria) and the micro- and mesofauna, of which protozoa and nematodes prevail, and therefore should not be neglected. Above all, protozoa can be expected to influence the activities and densities of the microflora components, or to be influenced by these decomposers since many protozoa are bacterial feeders (bactivorous). Despite the limitations connected to cultured techniques in order to identify and characterize environmental microbial communities, the relative estimates of some microbial groups before and after the passage of the wastewater through the reed beds, allow to assess the microbial removal efficiency of this system and will help to find out the trophic relationships between the prokaryotic and eukaryotic microbial community.

In this way the goal of this work was to assess the treatment efficiency in view of the sanitation quality of the effluent, and find a strategy to assess the food chain of this biocenose. The disclosures that may be "seen" in the reed beds' black box, may allow

finding means to optimize the systems' design in order to achieve better treatment efficiency and to explore the possibility of use some biocenose components as bioindicators.

METHODS

The CW of Salamonde II is *Águas do Ave*'s property. Its process diagram is shown on fig. 1. The treatment efficiency is monitored monthly by Águas do Ave: one-day compost samples of wastewater are collected at the entrance (A) – after screening– and at the system exit (F1 and F2) – compost samples are picked up at the two beds' outflows. Determinations of pH, five days-Biochemical Oxygen Demand (BOD_5), Chemical Oxygen Demand (COD) and Total Suspended Solids (TSS) were estimated on the compost samples A and F, by a recognized laboratory.
Aiming the understanding of the functioning of Salamonde's CW system, namely the behaviour of the reed beds and their role on the system, monthly sampling campaigns were planned, to gather simple samples from inlet and outlet of one bed of the system, in order to perform the traditional routine analyses and also, some microbiological ones. Beyond this assessment of the outflow' sanitary state of the system, additional substrate microscopic analyses were realized to understand the trophic relationships between bacteria and eukaryotic microrganisms. Inlet samples (IN) were gathered at the septic tank outlet (fig.1 - C) and outlet samples (OUT) were gathered at the outlet of the second reed bed of the system (fig. 1 - F2).

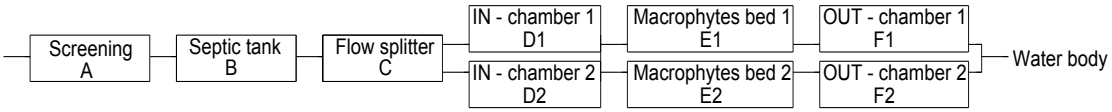

Figure 1: Process diagram of Salamonde II's CW.

Determinations of temperature, pH and redox potential took place *in situ.* These dependent variables were measured using a WTW pH-Electrode calibrated prior to measurements in accordance with manufacturer's instructions. The IN and OUT wastewater samples were collected into sterile glass bottles and kept cold on a thermo-box until analysed; analyses were done within 4 hours.
The laboratory analyses were performed at the Biology Department of University of Minho. IN and OUT samples were analysed for determination of five-days Biochemical Oxygen Demand (BOD_5, mg/l), Chemical Oxygen Demand (COD, mg/l) and Total Suspended Solids (TSS, mg/l). BOD_5 were performed by the respirometric method self-check measurement, a mercury-free pressure measurement that uses the OxiTop®

system.The determinations of COD were performed by the dichromate digestion method. The complete digestion of the samples was achieved on cuvettes with the reagents placed on a WTW thermoreactor a 148ºC for 120 minutes. The estimated values of COD (mg/l) were directly read on a WTW photometer (MPM 1500, WTW) with the appropriated wavelengths referred by the procedures instructions. Total suspended solids (TSS) were determined after filtration under vacuum, with 47-mm-diameter filters made of glass microfiber (934-AH Whatman) and after drying to constant weight at 105°C.

The Recovered Heterotrophics (RH), the pseudomonas (Ps) and total (TC) and fecal coliforms (FC) were the microbiological groups considered. The estimates of the microbiological groups were performed by the spread plate method, on selective or differentials media. A 0.1ml aliquot of selected dilutions of the sample was uniformly spread on top of the solid agar with the aid of sterile glass spheres. After incubation under specified conditions of temperature and growth time, the estimated numbers of the bacteria were obtained by counting the isolated colonies – colony forming units (CFU) that develop on top of the selected solid agars.

RH were recovered on the minimal R2A medium from Merck; the very good recovery capacity of bacteria in this medium is due to the large numbers of oligotrophic organisms in the environment that cannot be culture on rich media (Taylor *et al.*, 1983). The pseudomonas group was recovered both on Bacto Pseudomonas Isolation Agar (Pia) from Difco and GSP agar from Merck. This last medium is a complex one that allows the differentiation between large violet colonies of pseudomonas from large yellow colonies of Aeromonas, in spite of some background flora. The coliform bacteria were enumerated both in MacConkey agar, where the lactose positive bacteria develop red colonies and the lactose negative colorless and translucent colonies, and on Cromocult® agar. In this last medium it is possible to differentiate *E. coli* dark-blue to violet colonies, from salmon to red coliform colonies.

RESULTS AND DISCUSSION

Organic load parameters

The organic load parameters were determined by Águas do Ave (AA) using compost samples and by Universidade do Minho (UM), using simple samples (Fig. 2). Monthly samples from AA and UM corresponding to a given month were referenced with the same number, although not necessarily collected in the same day.

The IN-values of BOD_5, COD and TSS estimated in the compost samples show considerable variations, explained by the fact that these samples were collected before the septic tank. The values of the simple samples taken by UM are lower, and present

lower variations. This reflects the reduction and stabilization of the organic load achieved by the primary treatment. However, the values estimated for both OUT samples are very similar, thus showing the reliability of the simple samples.

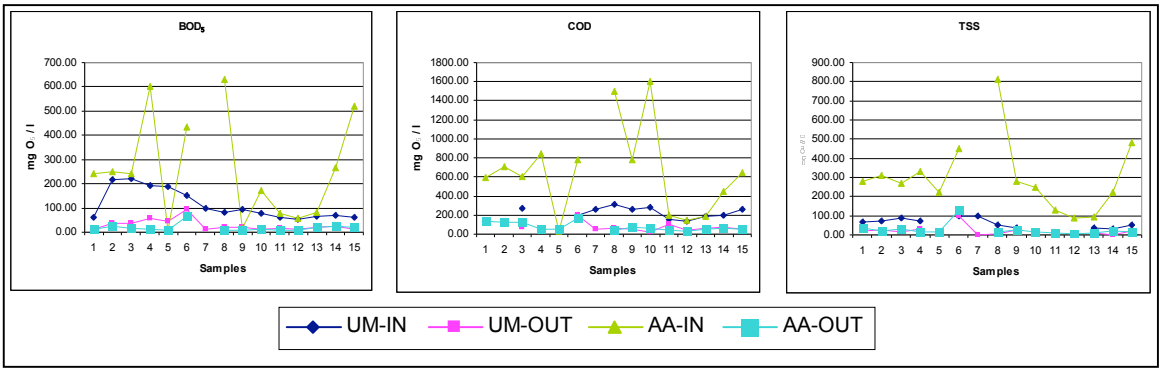

Figure 2: BOD_5, COD and TSS estimated in the compost samples (AA) and simple samples (UM) from IN and OUT.

The values estimated for BOD_5-OUT are always below the legal maximum value, with the exception of sample 6 (which will be discussed later). Those values were in average 29 mg/l for the simple samples and 15 mg/l for the compost samples. Similarly, the values estimated for COD-OUT are always below the legal maximum value, again except sample 6. Those values were in average 70 mg/l for the simple samples (n=11) and 76 mg/l for the compost samples (n=14). It should be mentioned that the difference in the number of samples corresponds to the 3 months in which the compost samples present the highest CQO values. Also the values estimated for TSS-OUT are always below the legal maximum value (except sample 6). Those values were in average 23 mg/l for the simple samples (n=11) and 27 mg/l for the compost samples (n=14).

In situ parameters

The sampling campaigns performed by UM included *in situ* determinations (Table I) of temperature (ºC), pH and redox potential (mV).

Table I: *In situ* determinations of temperature (ºC), pH and redox potential (mV).

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Temp. (ºC)	IN	8.5	7.6	7.8	12.7	15.7	17.6	22.9	22.3	21	15.3	8.2	9.3	8	8.3	16.8
	OUT	8.2	8.5	8.2	13	14.2	17.2	19.8	19.8	19.5	15.5	9.2	8.4	7.6	7.5	15.8
pH	IN	7.06	7.27	7.03	6.74	6.05	7.42	6.53	6.56	6.68	6.36	6.26	6.63	6.72	6.34	6.84
	OUT	6.26	6.64	6.37	6.50	6.08	6.36	5.76	5.83	6.23	4.75	6.08	6.30	6.65	6.10	6.00
P.Redox (mV)	IN	-13	-26	-9	5	46	-26	24	26	7	25	27	9	-2	22	17
	OUT	31	13	28	20	44	34	68	70	37	120	36	28	6	35	66

The temperature values were the expected according to the year season. The OUT-values were generally slightly lower than the IN-values, especially on summer months.
The pH variation from IN to OUT samples seems to indicate that the bed plays a part in the reduction of the wastewater pH, generally high in domestic effluents.
The evolution of the redox potential from IN to OUT samples may express the reduction ability of the substratum.

Microbiological Parameters

The average estimated values of the recovered microbiological groups on the solid culture media are show on Table II.
The average estimated value $1x10^6$ CFU/ml of RH on OUT-samples corresponds to a removal efficiency close to 90%. The correlation between IN- and OUT-samples (fig.3) shows an eight-times reduction of CFU/ml, setting apart the outlier value of the 13th sample.

Table II: Estimated values of Recovered Heterotrophics (RH), Pseudomonas (Ps), Aeromonas (Aa), Lactose positive bacteria (Lac+), Lactose negative bacteria (Lac-), *E. coli* and Colifoms, recovered on solid culture media.

Culture media	Microbiological group	Number of Samples (n)	IN - Sample ($CFUml^{-1}$)			OUT - sample ($CFUml^{-1}$)			removal efficiency (%)
			Average	Maximum	Minimum	Average	Maximum	Minimum	
R2A	HR	13	$1x10^7$	$3x10^7$	$1x10^6$	$1x10^6$	$4x10^6$	$4x10^3$	88
Pia	Ps	13	$9x10^5$	$2x10^6$	$1x10^5$	$1x10^5$	$7x10^5$	$4x10^2$	90
GSP	Ps	13	$4x10^5$	$2x10^6$	$8x10^4$	$4x10^4$	$2x10^5$	$3x10^2$	89
	Aa	13	$5x10^5$	$2x10^6$	$2x10^4$	$3x10^4$	$1x10^5$	$7x10^1$	94
	others	10	$1x10^6$	$3x10^6$	$4x10^5$	$7x10^4$	$7x10^1$	$2x10^5$	95
	Total CFU	10	$2x10^6$	$5x10^6$	$6x10^5$	$1x10^5$	$9x10^4$	$1x10^2$	96
McC	Lac+	13	$2x10^5$	$1x10^6$	$1x10^4$	$1x10^4$	$8x10^4$	$2x10^1$	95
	Lac^-	13	$2x10^5$	$5x10^5$	$3x10^4$	$2x10^4$	$1x10^5$	$7x10^1$	91
	Total CFU	13	$4x10^5$	$1x10^6$	$4x10^4$	$3x10^4$	$2x10^5$	$1x10^2$	93
CC	*E. coli*	11	$8x10^4$	$2x10^5$	$5x10^3$	$1x10^4$	$6x10^4$	$2x10^1$	91
	Coliforms	11	$3x10^5$	$8x10^5$	$7x10^2$	$2x10^4$	$8x10^4$	$2x10^2$	92
	others	10	$3x10^5$	$1x10^6$	$3x10^4$	$4x10^4$	$2x10^5$	0	88
	Total CFU	10	$7x10^5$	$2x10^6$	$3x10^4$	$6x10^4$	$3x10^5$	$1x10^3$	93

The recovered efficiencies of Ps IN-samples on Pia and GSP media were very variable, however it was generally four times higher on the former. The results obtained on OUT-samples were even more variable and in average six times higher on Pia. The greater specificity of Pia can explain this difference; in fact, 50% of the CFU recovered on GSP was background flora that made difficult not only the recovery of the target groups (Ps and Aa) but also their counting.

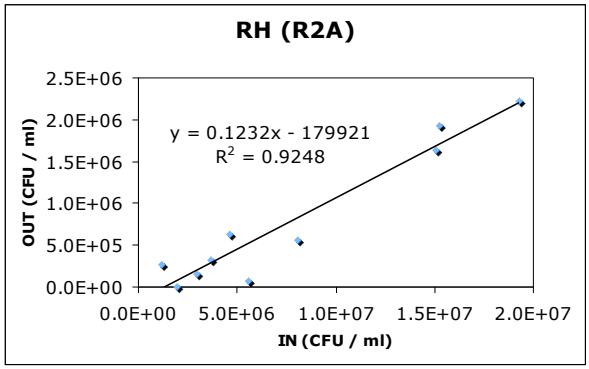

Figure 3: Correlation between HR (CFU/ml) recovered on R2A medium from IN- and OUT-samples ($p < 0.001$).

Comparing Ps' CFU with RH's CFU (fig.4) it seems that on IN-samples the numbers of Ps were about 10% of the RH. On OUT-samples the number of Ps was in average 6% of the RH, however this percentage is more variable (maximum 20%; minimum 0.5%).

Although the estimation of the coliform bacteria was about 3 times greater on CC medium than on McC, the removal efficiency of coliform bacteria - including *E. coli* (fecal coliform) - from wastewater was higher than 90%, except for sample 6. In this month these removal efficiencies were about 70%.

On IN-samples the R2A medium recovered twenty times more CFU than the McC medium, which may mean that 5% of RH were recovered on McC (fig. 4). On OUT-samples the CFU's recovered on McC were in average 2.5% of the RH, however this percentage is more variable (maximum 10%; minimum 0.07%).

Comparing total coliforms (TC – *E. coli* plus coliforms) recovered on CC with RH's CFU (fig. 4) it seems that on IN-samples the numbers of CT were about 3% of the RH. On OUT-samples the numbers of CT were in average 8% of the RH, however this percentage is more variable (maximum 32%; minimum 0.07%).

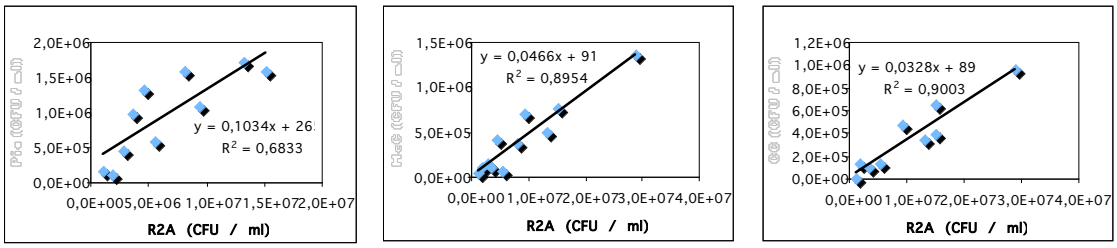

Figure 4: Correlations obtained from IN-samples between HR (CFU/ml) recovered on R2A medium and: (left) Ps (CFU/ml) recovered on Pia medium ($p<0.05$); (center) CFU/ml recovered on McC medium ($p<0.001$); (right) TC (CFU/ml) recovered on CC medium ($p<0.001$).

CONCLUSIONS

The concordance of the organic load data from compost and simple samples seems to express that simple samples are sufficiently representative to be used for the estimation of microbiological parameters. The possible relations of organic load with particular microbiological parameters additionally with the assessment of the substratum' microfauna may allow the establishment of bioindicators as monitoring tools to help improve the conditions of the system's performance, similarly to Sludge Biotic Index (SBI) by Madoni (1994).
Although the culture technique allows only to estimate a small fraction of the microbial communities really present (Baptista, 2003) and sometimes produces outlier results (detectable by the operator) the relative numbers show a coherent pattern: IN-samples exhibit some degree of proportionality between RH numbers of CFU recovered on R2A medium and CFU recovered on Pia, McC and CC, however, on OUT-samples the variability of recorded results express the unpredictable role of the unknown ecological relationships that take place on the CW biocenose. Nevertheless, the range of microbiological numbers at the bed outlet ensures the good sanitation quality of the treated wastewater. This is also supported by the atypical results obtained on month 6 when, due to the maintenance works on the CW the removal efficiency of all the parameters assess decreased. Therefore, these kind of monitoring programmes prove to be very important for the ecological study of these systems.

REFERENCES

Baptista, J. C. (2003). Microbial Communities in Subsurface Flow Wetlands. Proceedings of the 1st International Seminar on the use of Aquatic Macrophytes for Wastewater Treatment Constructed Wetlands, Lisboa, Portugal, 81-109.

Madoni P. 1994. A sludge biotic index (SBI) for the evaluation of the biological performance of activated sludge plant based on the microfauna analysis. *Water Res*. **28**, 67–75.

Seidel, K., Happel, H. and Graue, G. (1978). Contributions to Revitalisation of Waters. Published by Stiftung Limnologische Arbeitsgruppe, translated by Lillian Meetz, Krefeld-Hülserberg, 62PP.

Taylor, R. H. , M. J. Allen and E. E. Geldreich. (1983). Standard Plate Count: A Comparison of Pour Plate and Spread Plate Methods. Journal AWWA, *Research and Technology*, (Jan.) 35-37.