Extended abstract

Determining stoichiometric parameters in macrophyte beds with a fixed biomass respirometer

Susana Perdigão Ho

Instituto Superior Técnico – Universidade de Lisboa, Portugal

Abstract

Treatment wetlands (TW) are a low-cost and environmental technology for wastewater treatment and their increase interest has motivated substantial development in the models used to describe Nature-based processes to treat wastewater. However, the inevitable unpredictability of biological processes makes the modelling task challenging. Models for activated sludge (AS) have been successfully developed over the years, constituting a solid base for TW modelling. However, several authors have been presenting different insights and terminologies, and to this date a universal model capable of explaining the wide range of biological processes that take place in a TW is yet to be presented.

In an attempt to contribute to the current state of the art, the present work was developed with the purpose of assessing stoichiometric parameters of heterotrophic growth and storage yields of a lab-scale horizontal sub-surface flow (HSSF)TW with a fixed liquid phase – stationary gas flowing liquid (LSF) respirometer. This study focused on the characterization of a lab-scale TW (two types of medium beds with COD feeding concentrations of 800 and 1600 mgO_2/L), the construction and characterization of the respirometer, the respirometric tests with an acetate solution and their translation into oxygen uptake rate (OUR) curves for the calculation of heterotrophic growth yield, Y_H and storage yield, Y_{HSTO}, both in mg_{COD}/mg_{COD}.

It was found that the tested methodology was capable of presenting yield values for 18 respirometry tests and that those values were fairly in accordance with the literature, with average Y_H and Y_{HSTO} of 0,66 and 0,75 for beds fed with a COD of 1600 mgO_2/L and 0,67 and 0,83 for beds fed with a COD of 800 mgO_2/L.

Keywords: Horizontal sub-surface flow constructed wetlands; respirometry; stoichiometric parameters; biological modelling; sustainability

1. Introduction

Clean water is of the utter most importance to assure a proper quality of life. The increasing demographic trends and the fast pace of technological, industrial and agricultural development put tremendous pressure in responding to the amount of wastewater produced around the world every day.

As of 2016, it is reported that water pollution is seeing an increase since the 1990's in Latin America, Africa and Asia, mainly due to the growth in wastewater loadings to rivers and lakes. The most vulnerable populations to the consequences of water quality are women who are responsible for household chores which mainly involve the use of water, children who collect the water and often play near contaminated water sites, low income fishers and the low-income rural population that consumes the contaminated fish (UNEP 2016).

It is therefore necessary to enhance known and used models for wastewater treatment, as well as to come up with new ways of meeting this high demand. In this sense, the present thesis was developed with the aim of supplying experimental data to solidify the models so far designed for constructed wetlands – treatment wetlands (TW). This emerging technology basis itself on Nature and has shown its potential through effective results, low costs and low environmental impact. It is also of particular interest for application in developing countries' rural areas, where natural systems are preferred for economic and cultural reasons, opposed to traditional wastewater treatment plants.

The purpose of this thesis is the use of respirometry for determination of stoichiometric parameters in a lab-scale functioning horizontal sub-surface flow (HSSF) treatment wetland. Particularly, the aim of this work is the determination of values for heterotrophic growth and storage yield coefficients, Y_H and Y_{HSTO}, with the intent of pursuing the findings of Pisoeiro et al. (2017) in this field.

Given that respirometric techniques have so far mainly been used in activated sludge studies, several possibilities are still available for the application of this tool in treatment wetlands. For this reason, another of the objectives of the present work is to assess the viability of a liquid phase – stationary gas and flowing liquid respirometer, LSF, applied in the context of treatment wetland study.

Recent developments in modelling processes in treatment wetlands, such as the Constructed Wetland Model 1 (CWM1) also motivated the objective of supplying further experimental data for their consolidation.

2. Materials and methods

In order to supply new specific data to the current models used to describe kinetic processes in SSF TW – Constructed Wetland Model No1 (Langergraber et al. 2009) – a new adaptation of an LSF (liquid phase principle – static gas and fluid liquid) respirometer was developed for fixed biomass testing in a HSSF TW. By directly extracting and analyzing the biomass attached to the gravel medium, it was intended to maintain its conditions in the TW as much as possible, which in principle would avoid problems identified in the detached biomass procedure used by Pisoeiro et al. (2017). On the other hand, similarly to the work of Pisoeiro et al. (2017), the adopted procedures permit the *ex situ* analysis of a working HSSF TW.

Until present date, with the exception of Pisoeiro et al. (2017), the works carried out to the date of this one referred only to vertical flow TWs and to the use of LSF respirometers ((Andreottola et al. 2007), (Ortigara et al., 2011).

Between March and August of 2018, a series of respirometry tests with sodium acetate trihydrate solution substrate were carried out at the Environmental Laboratory of IST. For that, a respirometer system was assembled with the use of everyday lab materials, and sets of biomass were sampled from a HSSF lab scale TW located inside the lab (none of the sampled beds were planted). The DO readings and consequent calculation of OUR profiles allowed for the determination of the yield coefficient for heterotrophic bacteria, Y_H (mg_{COD}/mg_{COD}), as well as the associated yield storage coefficient for heterotrophic bacteria, Y_{HSTO} (mg_{COD}/mg_{COD}). Furthermore, the profiles obtained from the tests were classified according to the type of profile (I, II and III) proposed by Pisoeiro et al. (2017).

Characteristics of the lab scale HSSF TW sampled

The TW lab scale installation from which the fixed-biomass sample was extracted was setup in 2012 by Galvão and Matos (Galvão and Matos 2012) for the study of HSSF TW response to sudden organic load changes. The installation was composed of 9 PVC beds (B), 3 of which were continuously used in this present work – B2, B3 and B6. The beds that were fed with a higher synthetic sewage COD concentration are B2 and B3, 1600 mgO_2/L. B6 was fed with a lower concentration of 800 mgO_2/L. Table 1 summarizes the general characteristics of the beds.

Table 1: Characteristics of the lab scale HSSF TW samples

Dimensions of the plastic beds	1.1 m × 0.71 m × 0.76 m
Filling media: gravel	h = 30 cm, particle size, ps: 4-8 mm, porosity, p = 30%
Average water level below surface	5 cm

Description of the LSF respirometry system assembled

The system (Figure 1) was setup with conventional laboratory materials and was composed of a cylindrical PVC water tank, a plastic rectangular reactor closed with a plastic lid, a crystal pipe system for the water flow and two DO probes. The pipe system contained two plastic taps in the stretch between the pump and the rector: the first one as a water emergency exit, in case there were any clogging problems, and a second one for pulse acetate addition.

DO readings were performed at the inlet and outlet of the reactor, inside the water tank and immediately after the reactor, respectively. The DO inlet probe was placed in the water tank with a metal protection and the outlet probe was tightly enclosed in a glass cell supported by a claw, at the exit of the reactor. Also at the exit glass cell, a spongy filter was placed in the beginning of the exit pipe to prevent the biomass to enter the DO_{out} cell and cause false readings. The water tank contained a perforated pipe in the bottom connected to an air compressor to ensure oxygen levels close to saturation. A peristaltic pump allowed the water to circulate from the water tank through the system.

The DO sensors performed readings at different rates: DO_{in} values were recorded every 20 seconds and stored in the probe's memory, while DO_{out} values were registered and stored every second in a computer.
While running, the system would always be covered with a blackout curtain fabric to protect it from light exposure (which interferes with the probes' optical sensors readings), and to prevent contamination from external sources.

1 – water tank

2 – DO_{in} probe

3 – perforated pipe

4 – air compressor

5 – persitaltic pump

6 – tap for flow control

7 – tap for S_S addition

8 – reactor

9 – DO_{out} probe

10 – glass cell

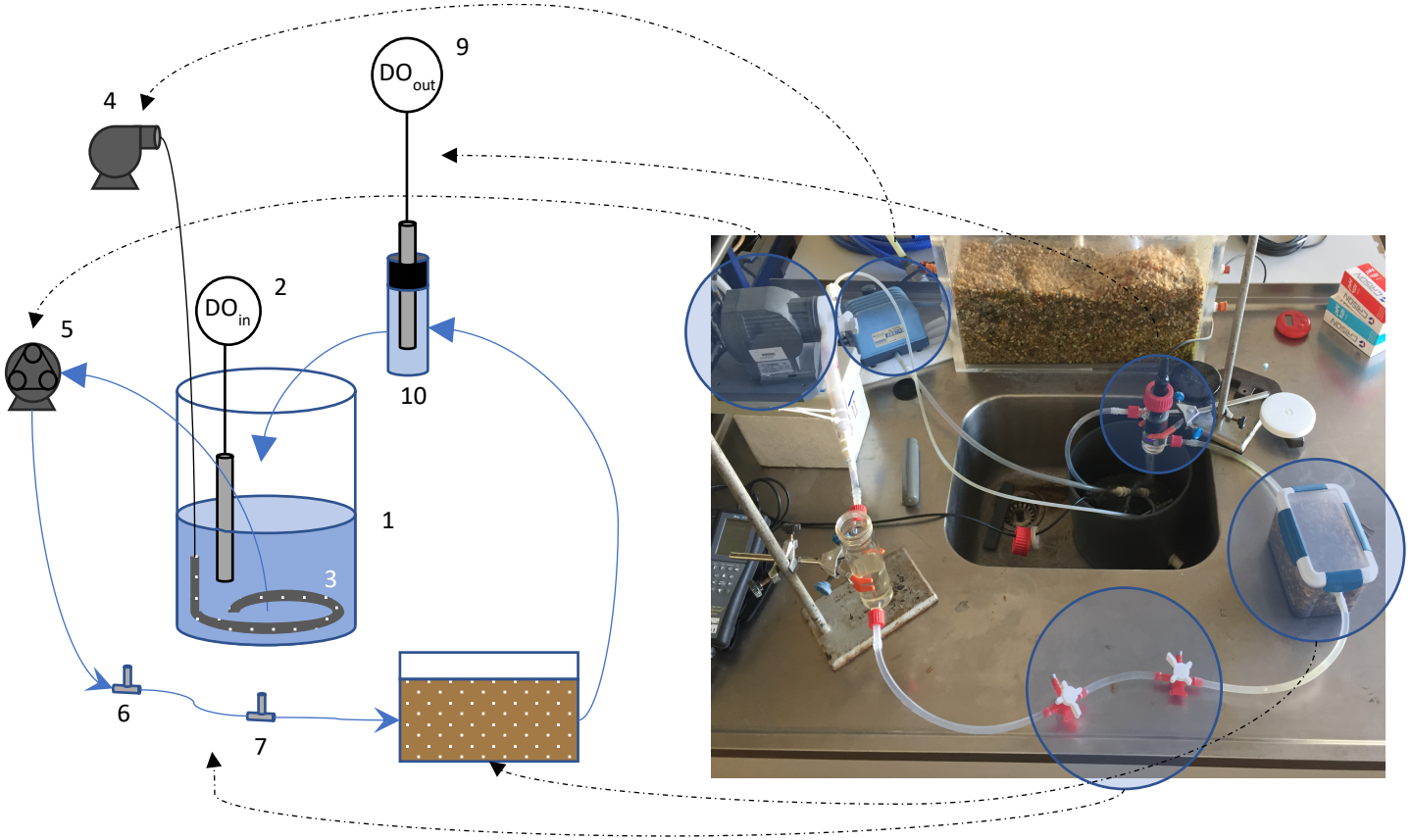

Figure 1: Scheme and setup of the LSF respirometer assembly

Respirometry tests with addition of pure substrate SS of sodium acetate trihydrate

In general terms, each set of respirometry tests was conducted in the following manner: Phase I – preparation of the system and establishment of the endogenous conditions; Phase II – set of respirometry tests with addition of a sodium acetate trihydrate solution; Phase III – determination of the sampled gravel's total and volatile solids

The solution of S_S is prepared with approximately 1 g of sodium acetate and 150 mL of distilled water. Two solutions were prepared during the experimental period: the first one prepared was used until the 28th of June and its COD was of 933,4 mg/L, while the second solution prepared was used until the 12th of July and had a COD of 961,4 mg/L.

For each set of tests, an approximate volume of 1400 cm^3 gravel media with the biomass in the form of a biofilm was extracted from a depth of approximately 10-15 cm of the bed (Figure 2). A small volume of water was carefully passed through the sample to assure no remaining substrate was present. The gravel was then transferred into the reactor and the system was filled with a total of 3L of tap water. DO recordings were then started once the flow at the outlet stabilized at around 16 mL/min. The system was left circulating until the DO_{out} recordings exhibited an endogenous level (1 to 2 days). Once this level was attained, a volume of 1 to 9 mL of the sodium acetate solution was added. The DO probes were left recording until the end of the test, indicated by the level of DO_{out} reaching again the endogenous level.

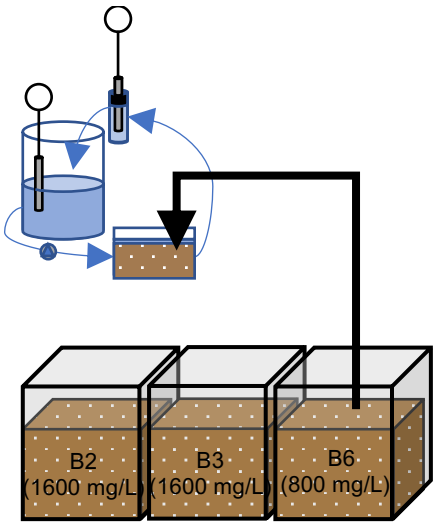

Figure 2: Bed sampling into the respirometer

3. Results and discussion

From DO readings to OUR profiles

In order to obtain the OUR profiles for each test, an Excel template with Visual Basics for Applications (VBA) programmed was used, where the DO inlet and outlet readings were inserted, along with other necessary variables, such as the volume of S_S (mL), the CQO of S_S (mg/L), and the porosity of the sampled gravel (30%).

Once the profiles were obtained, by visually identifying the key points of the test (Figure 3), a series of calculations were performed for the attainment of the desired parameters.

Respirometry with pure substrate results in one of two outcomes: a situation where there are no storage phenomena and one where these mechanisms take place. In case there is no storage, the oxygen consumption recorded by the probes (OUR profile) will only be due to the oxidation of substrate for growth of heterotrophic biomass. And so, the heterotrophic growth yield Y_H (mg_{COD}/mg_{COD}) is calculated with the integral (the area) comprised between the OUR line and the endogenous level. In a situation where storage is visually identified in the respirogram (green line in Fig. 3), a line is drawn from the point identified from the point of acetate depletion to the point where OUR returns to endogenous level. In this case, Y_H and Y_{HSTO} are calculated as depicted in Figure 3.

It is assumed that the consumption of stored products is linear for as long as there is acetate in the system. In this scenario, the heterotrophic growth yield Y_H is attributed to the simultaneous growth of heterotrophic biomass and the production of storage products by these bacteria.

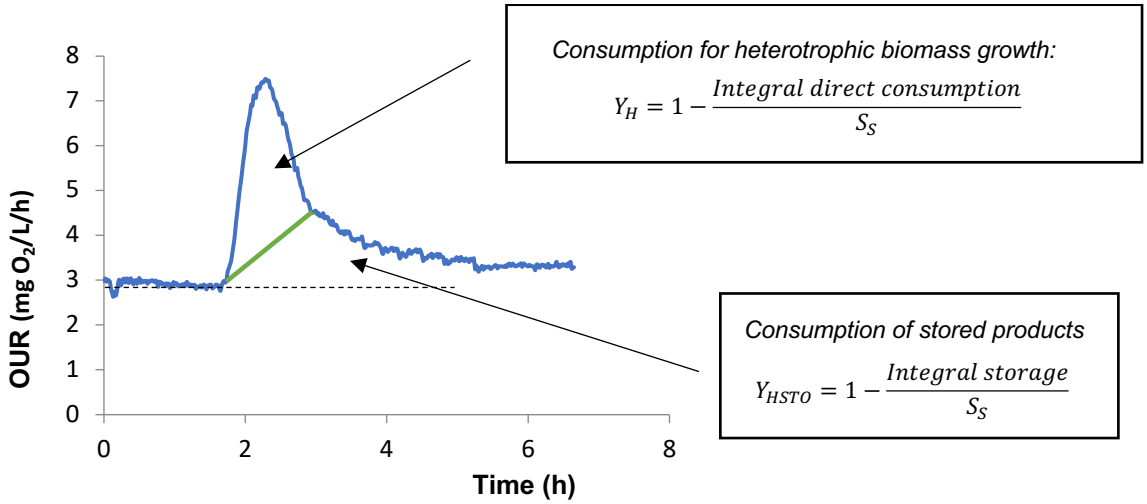

Figure 3: Visual identification of heterotrophic growth yield and storage yield and corresponding calculations

From the respirograms obtained in the present work, their respective growth yields Y_H and storage yields Y_{HSTO} were calculated as explained above, and the profiles were classified as I, II and III (Pisoeiro et al. 2017):

- Type I profiles are characterized by an immediate peak in the OUR after S_S addition, followed by the two different slopes corresponding to external substrate consumption and the slower consumption of stored products.
- Type II profiles present a less steep increase of OUR upon S_S addition, compared to the two other types of profiles, resulting in a smaller initial slope.
- Type III profiles are identifiable by a plateau that occurs after S_S addition, which is maintained until all of the acetate is consumed.

Sets of respirometry tests executed

For 6 months, from March to August, 18 successful respirometry tests with sodium acetate trihydrate solution addition were obtained. Of these, 8 were performed with samples from beds with a feeding concentration of 1600 mgO_2/L whilst 10 with beds of 800 mgO_2/L.

The obtained yield values from the tests performed were compiled in Table 2, where the beds were separated according to their concentration. For each test, the concentration of S_S in mg/L and the F:M ratio is also indicated.

The type of profile is classified according to Pisoeiro et al. (2017) and each test is identified by the number of the bed (B2, B3 or B6) and the number of the test ((1), (2), …) with the corresponding date.

Table 2: Y_H and Y_{HSTO} values and respective profile types obtained from respirometric tests performed to beds with concentrations of 1600 and 800 mgO_2/L

	Bed designation	Date of the test	S_S (mg/L)	F:M ratio	Type of profile	Y_H (mg_{COD}/mg_{COD})	Y_{HSTO} (mg_{COD}/mg_{COD})	Average Y_H	Average Y_{HSTO}
$B_{1600\ mg/L}$	B3 (1)	22-03-2018	7,6	0,78	I	0,78	0,85	0,66	0,75
	B3 (2)	23-04-2018	1,9	0,19	II	0,71	0,84		
	B2 (1)	25-05-2018	5,7	0,58	III	0,55	0,79		
	B2 (2)	14-06-2018	5,7	0,58	II	0,52	0,60		
	B2 (3)	19-06-2018	9,5	0,97	II	0,68	-		
	B2 (4)	26-06-2018	9,5	0,97	I	0,57	0,78		
	B2 (5)	27-06-2018	7,6	0,78	I	0,65	0,73		
	B2 (6)	02-07-2018	9,8	1,00	I	0,84	0,68		
$B_{800\ mg/L}$	B6 (1)	15-05-2018	9,5	1,36	I	0,55	0,82	0,67	0,83
	B6 (2)	15-05-2018	9,5	1,36	I	0,63	0,85		
	B6 (3)	16-05-2018	9,5	1,36	I	0,69	0,85		
	B6 (4)	16-05-2018	9,5	1,36	I	0,69	0,72		
	B6 (5)	17-05-2018	9,5	1,36	III	0,56	0,92		
	B6 (6)	04-07-2018	9,8	1,40	I	0,75			
	B6 (7)	05-07-2018	9,8	1,40	I	0,70	0,89		
	B6 (8)	11-07-2018	9,8	1,40	I	0,67	0,78		
	B6 (9)	12-07-2018	9,8	1,40	II	0,76	0,86		
	B6 (10)	12-07-2018	9,8	1,40	II	0,75	0,75		

In general terms, comparing the respirometry tests performed to the two types of beds, the results point towards a consistency regarding average values of Y_H for the two types of beds (0,66 for B_{1600} and 0,67 for B_{800}), which indicates that the feeding concentration of the bed does not impact the capacity of the heterotrophs in consuming the substrate for the initial growth phase. When comparing the average values for Y_{HSTO}, these were deemed higher in the case of the beds with a lower concentration, but still similar amongst the two types of beds (0,75 for B_{1600} and 0,83 for B_{800}).

The average values for Y_H and Y_{HSTO} calculated for the 17 tests (excluding test B2 (3) which did not present storage) were respectively of 0,67 (which is close to that indicated by the CWM1 of 0,63) and 0,79.

Even though test B2 (3) did not present storage, its Y_H (in this case, there was only direct S_S consumption for heterotroph growth) still resulted in a value within the average of the remaining tests.

It is also noteworthy how such different F:M ratios between B_{1600} and B_{800} resulted in similar yield average values. For example, an F:M ratio of 1,36 resulted in a Y_H of 0,55 and a Y_{HSTO} of 0,82 (test B6 (1)), while with an F:M ratio of 0,97, a Y_H of 0,57 and a Y_{HSTO} of 0,78 were obtained (test B2 (4)).

With the purpose of following the classification and interpretation of the types of profiles by Pisoeiro et al. (2017), Table 3 gathers the value ranges and average values per type of obtained profile in this study.

The average Y_H values for type I, II and III obtained by Pisoeiro et al. (2017) were of 0,67, 0,41 and 0,48, respectively. Comparing to those obtained in this study, the values for type I profile (0,68) were very similar, and for type III were also close (0,55). The biggest difference obtained was regarding average Y_H for type II (0,68). Overall, the average growth and storage yield values obtained for the three types of profiles were very similar to the ones by Pisoeiro et al. (2017), with the exception of average Y_H for type II profiles. The same can be said about the value ranges, which were also close to those by Pisoeiro et al. (2017).

Table 3: Y_H and Y_{HSTO} value ranges and average values according to each type of profile

Type of profile	no. of tests	Y_H (mg_{COD}/mg_{COD})		Y_{HSTO} (mg_{COD}/mg_{COD})	
		range	average	range	average
I	11	0,55-0,84	0,68	0,68-0,89	0,80
II	5	0,52-0,76	0,68	0,52-0,76	0,76
III	2	0,55-0,56	0,55	0,79-0,92	0,86

As far as the average values for Y_{HSTO}, the ones obtained by Pisoeiro et al. (2017) were of 0,83, 0,75 and 0,83, for types I, II and III. Compared to those of this study, they were in general very similar: 0,80, 0,76 and 0,86.
As in Pisoeiro et al. (2017), the values for Y_{HSTO} in this study were also higher than the Y_H values, indicating the similarity between these two methods for parameter assessment.

In general terms, the frequency of profile type occurrence was found to be similar to that obtained by Pisoeiro et al. (2017): type I profiles are the most common, followed by type II and the rarer type III.

Type I profiles represented the majority of the obtained respirograms, indicating that the general trend by the bacteria is to rapidly respond to the addition of substrate with a steep increase in the consumption of oxygen, followed by a relatively rapid decrease, while still allowing for the consumption of stored products.

The less frequent occurrence of type III profiles indicates that the behaviour of simultaneous growth and substrate storage reflected by the OUR plateau after the peak of S_S addition is not the most common situation verified. For this study, it was found that the higher average values of Y_{HSTO} correspond to this type of profile. Given the low frequency of these profiles, it is difficult to make more accurate remarks concerning typical profile III behaviour. However, the kinetic of this type of profile are that of an immediate response to the S_S addition and of a situation where the heterotrophs are at their maximum consumption during the test, given that there is no shift from feast to famine conditions. Because these were not the conditions sustained during the testing period, the low frequency of occurrence of this type of profiles was expected.

It should be mentioned that the visual resemblance and the similarity in average values and value ranges between profiles of type I and II leads to some difficulties in distinguishing them, which might explain the high count of type I profiles.

Out of the 18 OUR profiles obtained, only one did not reveal storage (test B2 (3)). For the test B6 (6), even though storage is identified visually on its respirogram, the value obtained of Y_{HSTO} was higher than 1,0 and for this reason, it was discarded.

More correlatable values and clearer patterns were expected to be observed with the use of this LSF respirometer, given that it would overcome certain difficulties, such as that of the attachment of the suspended biomass to the probes in the case of the LSS respirometer used by Pisoeiro et al. (2017). Also the attempt to replicate the same conditions (flow, S_S amount, temperature and others) for consecutive tests was expected to produce more similar yield values, which was not observed. For this reason, it is also suggested the study of consecutive respirometric tests, in order to attempt at standardizing the behaviour of active biomass, providing greater robustness to the models under development.

4. Conclusions

A lab-scale HSSF TW replicated as beds (B) fed with different synthetic sewage concentrations (1600 mgO_2/L and 800 mgO_2/L) was successfully sampled and tested with a fixed biomass respirometer – LSF setup – for the assessment of the stoichiometric parameters of heterotrophic growth yield, Y_H and storage yield, Y_{HSTO}, both in mg_{COD}/mg_{COD}. Respirometric tests with addition of readily biodegradable substrate in the form of a sodium acetate trihydrate solution resulted in the average Y_H value of 0,66 and 0,67 for B_{1600} and B_{800}, while the average values of Y_{HSTO} were of 0,75 for B_{1600} and of 0,83 for B_{800}. There were no significant differences in the yield values for different F:M ratios. The average Y_H value of the 18 tests, 0,67, is consistent with that of 0,63, proposed in the general description of the CWM1 (Langergraber et al., 2009).

The classification of the OUR profiles according to the proposed classification by Pisoeiro et al. (2017) of types I, II and III was successfully applied, presenting similarity in the results and indicating thus semblance between the calculation method used in this work and that of the mentioned authors. Particularly, it was found that the majority of the profiles is of type I, where the biomass responds with a peak in the OUR upon Ss addition, correspondent to the initial growth yield, with a subsequent observable behaviour of consumption of stored products. The rare occurrence of type III profiles motivates further studies to supply with more data to the proposed classification.

The use of the LSF respirometer, which proved to be easily assembled with common laboratory materials, has shown promising results when it comes to studying an operating TW, with *ex-situ* analysis made possible by sampling the mentioned wastewater treatment system. Nonetheless, the variability of the obtained results and the difficulty in correlating them to the F:M ratios or the type of profile obtained still encourages the necessity of respirometric techniques paired with stronger confirmation procedures, such as pH control and more focused food-to-microorganism ratio considerations.

Storage evidence was very strong with the performed tests, where out of 18, only 1 did not present such behaviour. This observation supports the need for further work to be conducted regarding the storage mechanisms that have been explored in the last two decades with the development of models for activated sludge and, more recently, constructed wetlands.

5. Bibliographic references

Andreottola, G., E. Oliveira, P. Foladori, R. Peterlini, and G. Ziglio. 2007. “Respirometric Techniques for Assessment of Biological Kinetics in Constructed Wetland.” *Water Science and Technology* 56 (3): 255–61. https://doi.org/10.2166/wst.2007.512.

Galvão, Ana, and José Matos. 2012. “Response of Horizontal Sub-Surface Flow Constructed Wetlands to Sudden Organic Load Changes.” *Ecological Engineering* 49: 123–29. https://doi.org/10.1016/j.ecoleng.2012.08.033.

Langergraber, Guenter, Diederik P L Rousseau, Joan García, and Javier Mena. 2009. “CWM1: A General Model to Describe Biokinetic Processes in Subsurface Flow Constructed Wetlands.” *Water Science and Technology* 59 (9): 1687–97. https://doi.org/10.2166/wst.2009.131.

Ortigara, A. R C, P. Foladori, and G. Andreottola. 2011. “Kinetics of Heterotrophic Biomass and Storage Mechanism in Wetland Cores Measured by Respirometry.” *Water Science and Technology* 64 (2): 409–15. https://doi.org/10.2166/wst.2011.547.

Pisoeiro, J., A. Galvão, H. M. Pinheiro, F. Ferreira, and J. Matos. 2017. “Determining Stoichiometric Parameters of Detached Biomass from a HSSF-CW Using Respirometry.” *Ecological Engineering* 98: 388–93. https://doi.org/10.1016/j.ecoleng.2016.07.003.

UNEP. 2016. *A Snapshot of the World’s Water Quality: Towards a Global Assessment*. https://doi.org/978-92-807-3555-0.