Determination of stoichiometric parameters in respirometric tests from full-scale operating HSSF constructed wetlands

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Abstract

This research studies microorganisms' activity in biofilms from a full-scale operating horizontal subsurface flow constructed wetland (HSSF-CW) treating municipal wastewater. It thereby transposes previous lab-scale work to a full-scale HSSF-CW with soil media and *Phragmites australis*. The aim is to establish a better understanding of the internal processes and to develop stoichiometric parameters for modelling. Ten samples, manually obtained across the bed, were placed inside an LSF-respirometer (Liquid phase, Static gas, Flowing liquid) which allows testing aerobic substrate biodegradation. The respirometer includes a closed box with the sample wedged between two vertical gravel layers. During the tests DO-concentrations of the recirculating water from an aerated tank placed before the inlet are measured together with those of the outlet of the box. Most of the respirograms (12) obtained had a peak value followed by a decrease in OUR and 7 profiles revealed slower reactions to substrate additions. Only six of the respirograms, corresponding to the inlet and outlet of the wetland, had a plateau of maximum OUR. Average results for Y_H were measured as 0.852 mg COD/mg COD and for Y_{STO} as 0.895 mg COD/mg COD, with ranges of 0.324-0.961 and 0.794-0.968 mg COD/mg COD respectively. High yield values are probably caused by a partial adsorption of substrate on the soil. Extremely fluctuating results were observed. Based on the hydraulic retention time and volatile attached solids a potential preferential flow path is assumed at the right hand side of the CW with a clogged area at the centre.

Keywords: Constructed wetland; respirometry; growth yield; storage mechanism; OUR-profile.

1. Introduction

In order to contribute to a sustainable way of living, development of technology offering ecological solutions should be encouraged. Constructed wetlands are a low-cost, eco-friendly alternative to use as a natural wastewater treatment system.

To help modern developments of constructed wetland systems, this research studies the microorganisms activity in biofilms so stoichiometric parameters could be determined as the heterotrophic growth yield coefficient values (Y_H and Y_{STO}). It thereby transposes the work of Ho (2018) from a lab-scale to a full scale operating wetland bed.

The obtained results could provide better insight into the different growth patterns of microorganisms throughout the wetland bed. In addition, this research aims at providing data for modelling tools of constructed wetlands as the Activated Sludge Model 3 (ASM3) (Henze et al., 2000), Simultaneous Storage And Growth model (SSAG) (Hoque et al., 2008) or Constructed Wetland Model 1 (CWM1) (Langergraber et al., 2009).

1.1 HSSF constructed wetland

Constructed wetlands, or human imitations of natural '*swamps*' are mainly used as a tool in wastewater treatment technology. Especially carbonous compounds (COD/BOD) will be reduced by microbiological activity inside the wetland, but also nitrates and phosphates, solids and other components will be removed partially. In addition it could help control floods and create different habitats. Nevertheless, it takes a lot of space (Rousseau, 2017).

Different classifications of wetlands can be made depending on the water flow, e.g., free water surface (FWS), horizontal subsurface flow (HSSF), vertical flow (VF) or a combination.

This research studies the growth of microorganisms from a full-scale HSSF constructed wetland with soil as filling media (Kickuth system), treating municipal wastewater and planted with *Phragmites australis* (Brix, 2003).

The investigated wetland is located in Barroca D'Alva, 20 km from Lisbon. Wastewater treatment is achieved by a septic tank followed by 4 wetland beds operating in parallel.

A typical HSSF constructed wetland, as represented in figure 1, usually consists of an distribution system for the influent, followed by an horizontal flow of the wastewater through the filling media and vegetation roots where the treatment processes take place. The effluent leaves the system at the bottom-end of the wetland (Dotro et al., 2017).

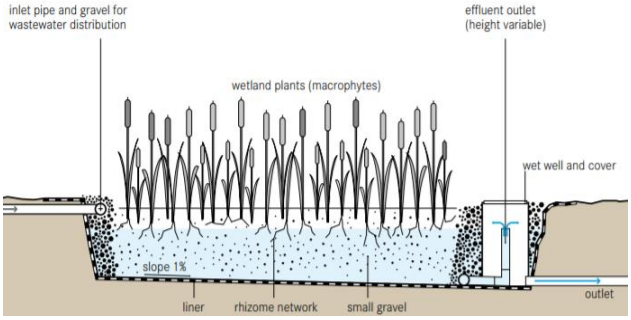

Figure 1: HSSF constructed wetland

The water level is often held below surface of the media to exclude the possibility of a free water surface flow. Due to the lack of aeration and light inside the wetland, most biological processes are anoxic and anaerobic (Dotro et al., 2017). Nevertheless, (facultative) aerobic microorganisms still contribute significantly to the wetlands' ecosystem and are considered in modelling studies.

The choice of the right type of filling media is essential in the design of a constructed wetland. It has a large influence on the hydraulic pathways, the hydraulic retention time, the filtration of solids or the adsorption of substrate into the pores. Especially in terms of clogging it is important to have the best suited media present in the wetland or to react quickly if clogging takes place (Dotro et al., 2017).

The most common macrophyte species are the *Phragmites australis*. The green leaved plants with a upright stem are typically known for its invasive character. Nevertheless often chosen because of the excellent photosynthesis rate and nitrogen- and carbon uptake. The roots nestle itself in the soil so well, it helps controlling the water level, i.e., flood controlling (Brix, 2003; Chambers et al., 1999; Harrison, 2016). Furthermore, the presence of small aerobic zones is supposed just near the roots of the vegetation. Atmospheric oxygen is inserted into the system via the stems and leaves to the roots (Brix, 2003).

1.2 Respirometry

Respirometry is a term used to describe the measurement of oxygen consumption. The intercellular biochemical processes, also called cell respiration, comprises the substrate consumption by a redox reaction. A reaction where the electron donor (organic or inorganic compounds) and the electron acceptor (O_2, NO_2, NO_3^-, SO_4^{2-}) produce ATP molecules. This is subsequently used as energy source for biomass growth and cell maintenance.

By controlling certain conditions inside a reactor, the precise amount of oxygen consumption can be determined. This is related to the mentioned biochemical processes of the microorganisms. The result of respirometric measurements can be expressed in terms of the Oxygen Uptake Rate or OUR, which represents the speed of oxygen consumption and is linked with the different stages of the cell respiration (Vanrolleghem, 2002).

The respirometric tests are executed with box reactors, calling respirometers. With these reactors, the exact oxygen consumption for a specific period of time can be measured. A respirometer is mostly occupied with sample, dividing the space in a part as gas phase and a part as liquid phase. Different type of respirometers exist depending on the oxygen measurements in the gas- or liquid phase and static / flowing streams. Within the framework of this research a Liquid phase, Static gas, Flowing liquid reactor is used, i.e., an LSF respirometer (figure 2). For these type oxygen transfer between the two phases is absent (Galvão, 2017; Vanrolleghem, 2002).

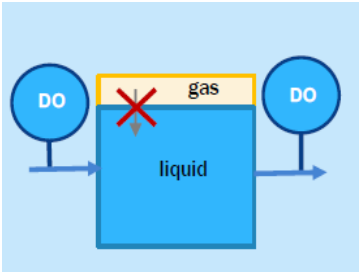

Figure 2: LSF-respirometer (Galvão, 2017)

1.2.1 Respirogram and storage mechanism

Previous work of Pisoeiro *et al.* (2017) and Ho (2018) observed the presence of the storage mechanism. This describes the reaction of microorganisms regarding the feeding pattern. At stages with an excess of oxidizable components, i.e., feast conditions, the organisms will convert the substrate to energy for cell maintenance and -growth. However, a fraction of this energy is used for the production of storage products. These storage products mostly consist of polysaccharides and lipids. At famine conditions, the stored products are oxidized for cell growth or -maintenance (Pisoeiro et al., 2017).

Following the simultaneous storage and growth model (SSAG), the use of energy for cell growth due to direct

substrate oxidation takes place simultaneously with the use of energy for storage production. This vision is a extension of the existing ASM3, which considered growth and storage as two separate phases. At this point, no verified hypothesis is accepted concerning the distribution between the growth and storage formation during feast conditions. Therefore a linear contribution is assumed, represented by the line between the green and yellow point at figure 3 (Hoque et al., 2008).

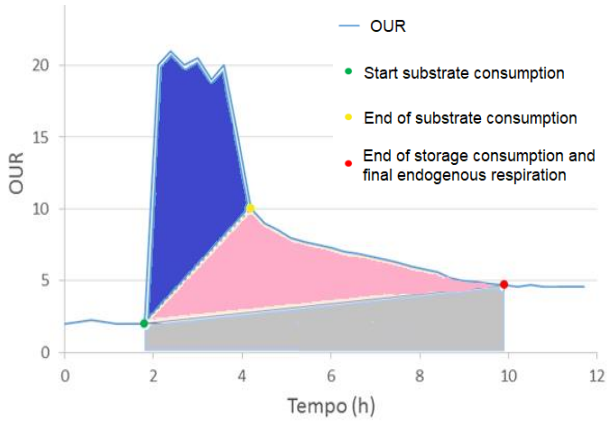

Figure 3: Division of the OUR-profile in (blue) direct cell growth, (pink) storage products formation and consumption, (grey) endogenous respiration level (Pisoeiro et al., 2017)

Endogenous respiration is defined as the state where microorganisms consume oxygen for oxidation reactions. The produced energy is subsequently used to maintain their vital processes. Theoretically the amount of cells remain constant with a level of endogenous respiration in equilibrium, i.e., equal cell growth and –decay (Hao et al., 2010). Oxygen concentrations higher than 2 mg O_2 / l are required in order to avoid oxygen-limiting (anaerobic) regions (Spanjers et al., 2016).

Figure 3 illustrates the OUR progress in function of the time. The respirogram starts with an initial constant OUR-level (endogenous respiration) until substrate addition takes place. This causes a very sharp peak of the DO-consumption. Once the availability of readily biodegradable organic substrate is ending, the profile makes a fast decrease until all substrate is depleted and consumption of the stored products starts. This is characterized by a slow decrease of the OUR profile until it ends back in balance at endogenous respiration (Ortigara et al., 2011).

Pisoeiro *et al.* (2017) introduced three different types of respirograms measured from the biomass.

- Type I respirograms are described as a standard curve with a high initial peak after substrate addition, rapidly followed by a fast decrease and later with a slower decrease quintessential for the storage phenomenon.
- Type II respirograms start with a significant slower OUR-slope as a slow starter. When reaching the maximum OUR-value, a similar gradient takes place as with type I. However, tests with absence of the storage mechanism are common.
- Type III respirograms are similar to the first one, but remain a more constant ‘plateau’ after reaching the maximum OUR. This typical respirogram is presumed to characterize biomass at the wetland subjected to high loading rates.

The use of modelling tools was first introduced with the ASM1, which evoluated to the more extensive ASM3 such as the introduction of storage products, a different view on nitrogen and phosphorus removal, etc. In this model the cell growth on substrate oxidation and the cell growth on formed storage products occur not simultaneously, which is in contrast with the more recent SSAG model as a modification of the ASM3 (Henze et al., 2000; Hoque et al., 2008). Furthermore, the CWM1 is one of the most modern and accurate models. But, the use of it in respirometry would be more difficult because of the absence of the storage phenomenon (Langergraber et al., 2009).

1.2.2 Process parameters and interferences

During the measurements using respirometry, several parameters could influence the process. By measuring the temperature, a correction for the OUR profile can be calculated. Nevertheless, a fluctuating temperature could also accelerate of delay the microbiological reaction rates or could influence the oxygen solubility in the water (Vanrolleghem, 2002).

Ambient light could initiate photosynthesis inside the reactor which is undesirable. Because of the strong light-dependency of the luminescence based DO-probe, all ambient light should be prevented as much as possible.

With a low flow rate possible anaerobic zones could occur (Spanjers et al., 2016). With higher flow rates, an increased wash out of the biomass or added substrate could be caused. Therefore a balanced, continuous flowrate is important (Spanjers et al., 2016).

Furthermore the managed F:M-ratio should be adjusted to cause a clear reaction of the microorganisms after the addition of acetate. The used acetate acts as a readily available organic compound (BOD) without any present nitrogen inducing nitrification. In that way the most optimal circumstances were used to measure the typical respirograms as shown in figure 3.

Measurements of the systems water volume, sample volume and weight are essential in further parameter

determination or to detect any dependency between different parameters.

Also large changes in pH could indicate several internal changes or interferences (Gernaey et al., 2001).

A common interference is the presence of nitrification reactions during the tests, this influences the total amount of oxygen consumed. If present, nitrification processes would be clear at the respirograms characterized by different plateaus of nitrification (Ortigara et al., 2011). To prevent nitrification, several inhibitors could help. Due to the rather absence of nitrification during the research of Ho (2018), inhibitors were not used during these tests.

Sulphur and iron bacteria oxidizing H_2S to SO_3^{2-} and Fe^{2+} to Fe^{3+} could also cause dissolved oxygen (DO) changes. However, it would have less influence if present in the biomass (Vanrolleghem, 2002).

2. Materials and methods

2.1 Sampling methodology

In order to obtain stoichiometric results from a full-scale operating wetland with soil filling media, the wetland of 20.3 m x 20.8 m (width-length) got divided in 3x3 intersections with the aim of collecting 9 samples from diverse areas of the field. One extra sample was collected due to unexpected difficulties regarding the hydraulic retention time of several samples.

When extracting the soil from the wetland an 'extraction tube' provided with notches at the bottom side was partly inserted into the soil. Using a small shovel obstructing roots were cut, so samples could be collected from a consistent and desired depth of 15-20 cm. The soil was gathered by hand to prevent heavily compression of the samples. Large pieces of roots or stones were filtered out as much as possible.

2.2 Respirometer setup

The used LSF-respirometer, based on the studies of Ho (2018), was assembled and used at the environmental lab of Instituto Superior Técnico (ULisboa), in Lisbon. The respirometer (figure 4) mainly consisted of an aeration tank where the DO of the oxygen-saturated water is measured, followed by the reactor (box) provided with two gravel layers and the soil sample. Subsequently, the oxygen concentration is measured again at the outlet of the reactor. Water is continuously circulated at the system. An emergency exit was implemented in case of rising water levels. Substrate addition got realized at a second 3-way valve.

The reactor consisted of a 16 x 10.5 x 11 cm plastic box with an airtight lid. It was adjusted compared to the system used by Ho (2018). A first thin gravel layer was applied to improve the water distribution at the inlet. The second (larger) layer at the outlet of the box acted as a natural filter for dirt an small solids. In this way more stable signals in the measuring cell were maintained by the reduction of turbidity. Further, this last layer helps decreasing the overall pressure head for the box. This would contribute to a lower possibility of an obstructed system.

When running the respirometry test, the box got filled for approximately 80 % with the soil samples, remaining with 20 % of air.

2.3 Respirometry test procedure

Preparation: In order to maintain an accurate respirometry test, with changing sample every part of the setup was cleansed and disinfected with water, soap and/or bleach. Different DO-probes used at the tests were calibrated regularly. DO variations of maximum 1 g O_2/L were measured and used in a correction. COD content was measured of the prepared 0.05 mol/l sodium acetate solutions, i.e., the external substrate. Two solutions were prepared during the research with 964 and 2909 mg COD/L respectively.

Test procedure: When introducing the soil into the reactor, the exact mass of the sample was weighted. At the start of a fresh sample, the system was kept running for 48 hours approximately with water not recirculating through the system, but rinsing the soil. This resulted in a discharge of an excess of dirt and tiny solids. Also the microorganisms present in the sample were able to reach an equilibrium at endogenous respiration level.

Once endogenous respiration level was reached and the DO-signal was constant, addition of the substrate could take place. Important points of attention are the absence of leaks, contamination or interferences. The determination of the water flow rate was performed a couple of times during one test. Within this complete process the changes in DO concentrations were recorded and plotted in a graph.

After completing several respirometric tests with the sample, a tracer test was performed to determine the exact hydraulic retention time (HRT). For this procedure, the 'step-change integral modelling methodology' was performed using the work of Bonner *et al.* (2017).

At last, measurements for the dry matter (DM) and volatile attached solids (VAS) were realized (EPA, 2001).

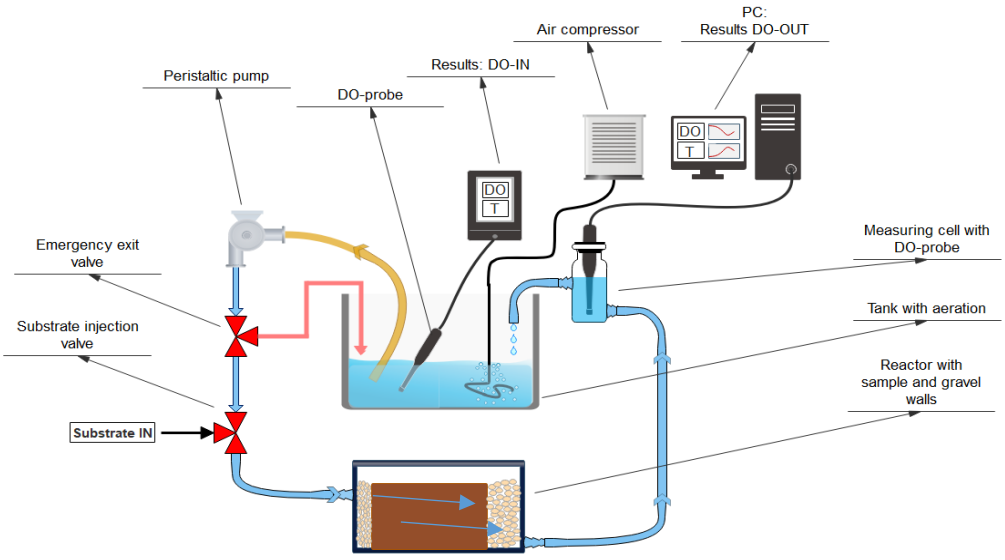

Figure 4: Representation of the LSF respirometer test setup

2.4 Yield coefficients calculation

The Oxygen Uptake Rate (OUR) represents the rate at which oxygen is consumed by calculating the DO difference of the incoming and outgoing water, divided

by the HRT. The result typically fluctuate at some points due to the temperature changes. These fluctuations were corrected by equation (1).

$$OUR_{corrected} = OUR * \alpha^{20-T} \quad (1)$$

With 20°C chosen as reference temperature, T in actual temperature (°C) and α as correction factor (= 1.08) (Ortigara et al., 2011).

When creating the respirogram, points for the substrate addition, substrate depletion and end of storage products oxidation are determined visually. These points are set on the visual changes in the gradient and represent the green (t_0), yellow (t_1) and red (t_e) points respectively (figure 3).

The oxygen consumption for direct growth on substrate oxidation is related with the blue area at figure 3. The corresponding growth yield, Y_H in mg COD biomass/mg COD biomass, is calculated with equation (2).

$$Y_H = 1 - \frac{\int_{t_0}^{t_1} Substrate\ consumption}{Feeding} \quad (2)$$

The storage yield, Y_{STO} in mg COD storage/mg COD biomass, corresponding with the pink area in figure 3 is calculated by equation (3).

$$Y_{STO} = 1 - \frac{\int_{t_0}^{t_e} Storage\ consumption}{Feeding} \quad (3)$$

With the feeding (S_S) as the total concentration added sodium acetate (mg COD/L). The integral represents the oxygen consumption for its specific region. So both of the integrals above include the subtraction of the area representing the endogenous respiration from the total integral of the curve.

3. Results and discussion

3.1 Tracer tests and hydraulic retention time

With every changing sample, the HRT was measured based on the step-change integral modelling methodology from Bonner et al. (2017). Results do not represent the hydraulic conductivity inside the wetland bed, but show the HRT inside the respirometer. However, the HRT may be linked to the hydraulic conductivity or density at the different wetland areas. Values for the HRT may be overestimated due to the two graver layers inside the respirometer box. Results are tabulated in table 1.

The HRT increases with the wetland length. This is unexpected as water flowing through a wetland bed is naturally filtered from solids. When reaching the end of the bed, less solids should be present to obstruct soil

Table 1: Summary of results for the respirometry tests

	Y_H (mg COD/mg COD)	Y_{STO} (mg COD/mg COD)	Type	S_S (mg/L)	HRT (min)	VAS (g/L)
Sample 1*	0.885	0.885	I	15.376	27	44.459
Sample 2	0.629	0.847	III	32.877	36	131.343
Sample 3	0.938	0.968	I	34.399	27	47.830
Sample 4	0.929	0.933	I	31.62	34	121.901
Sample 4A	0.769	0.794	II	34.545	53	41.969
Sample 5	-	-	-	-	-	22.970
Sample 6	-	-	-	-	22	43.671
Sample 7	0.961	0.962	II	30.302	47	36.156
Sample 8	0.324	0.875	III	32.322	46	34.515
Sample 9	-	-	-	-	47	33.099

** With sample 1, 2, 3: first row right, middle, left respectively*
sample 4, 4A, 5, 6: second row, right, middle-right, middle, left respectively
sample 7, 8, 9: third row, right, middle, left respectively

pores and to make the HRT rise.

Secondly, the results show higher values near the centre of the bed, which could indicate a clogged zone. Due to a the very dense structure, no tests were performed with sample from point no. 5 as it flooded with every attempt to get the water flowing through.

The HRT at sample point 6 is assumed to be incorrect due to preferential flow paths inside the reactor. During the tests, only very low flow rates could be maintained in order to prevent clogging. So a high HRT was expected.

3.2 Dry matter and volatile attached solids

Values for the dry matter content and volatile attached solids (assumed to be linked with the amount of biomass) were determined using a thermal process described by EPA (2001).

For all sample points at the wetland bed the dry matter content showed results within the range of 61.62 % - 78.13 %..

Regarding the VAS, the amount of biomass reduces slightly with the wetland beds' length. This is probably caused by the lower amount of food available at the end of the wetland because organic matter is mostly oxidized at the beginning of the bed (table 1).

At sample point numbers 2 (wetland inlet) and 4 the VAS is extremely high, which indicates a significant higher amount of biomass present in these areas (table 1). Also at the supposed clogged centre, the concentration of VAS is at is minimum. This confirms the hypothesis of a large preferential flow path at the right-hand side of the wetland and a lower hydraulic conductivity at the left-hand side of the bed. However, a higher amount of biomass would be expected at the outlet of the wetland (no. 8), but this could be due to the efficient BOD removal at the start of the wetland bed.

3.3 Yield coefficients and parameters

From all sample points, 25 different tests were performed. For sample point no. 5, no results were obtained due to the problem to start the test. Samples 6 and 9 were used in different tests, but showed very abnormal results and fluctuating OUR-profiles. Therefore these tests were excluded in the summary of results for the calculated yield coefficients.

For every test, the feeding concentration (S_S) was held around 30-40 mg/L. The temperature was measured in order to use as a correction for the OUR-profile. The range of average temperatures in the system measured go from 13.83 °C to 17.92 °C. Average results for every sample point are summarized in table 1. No large variances were observed between different tests with the same sample.

A couple of respirograms from the measured samples are displayed in figure 5. The two graphs represent the measured and the temperature corrected OUR-profile, the red marks indicate the time of substrate addition, substrate depletion and the end of each test. Different types of respirograms were obtained, with varying values for the level of endogenous respiration, test duration and maximum OUR peak height.

From the values in table 1, the average Y_H is 0.852 mg COD/mg COD if an outlier at sample 8 was excluded. With this outlier the average lays at 0.776 mg COD/mg COD. The range for the growth yield directly on substrate is 0.324 – 0.961 mg COD/mg COD. For the storage growth yield, Y_{STO} the average is 0.895 mg COD/mg COD with a range of 0.794 – 0.968 mg COD/mg COD.

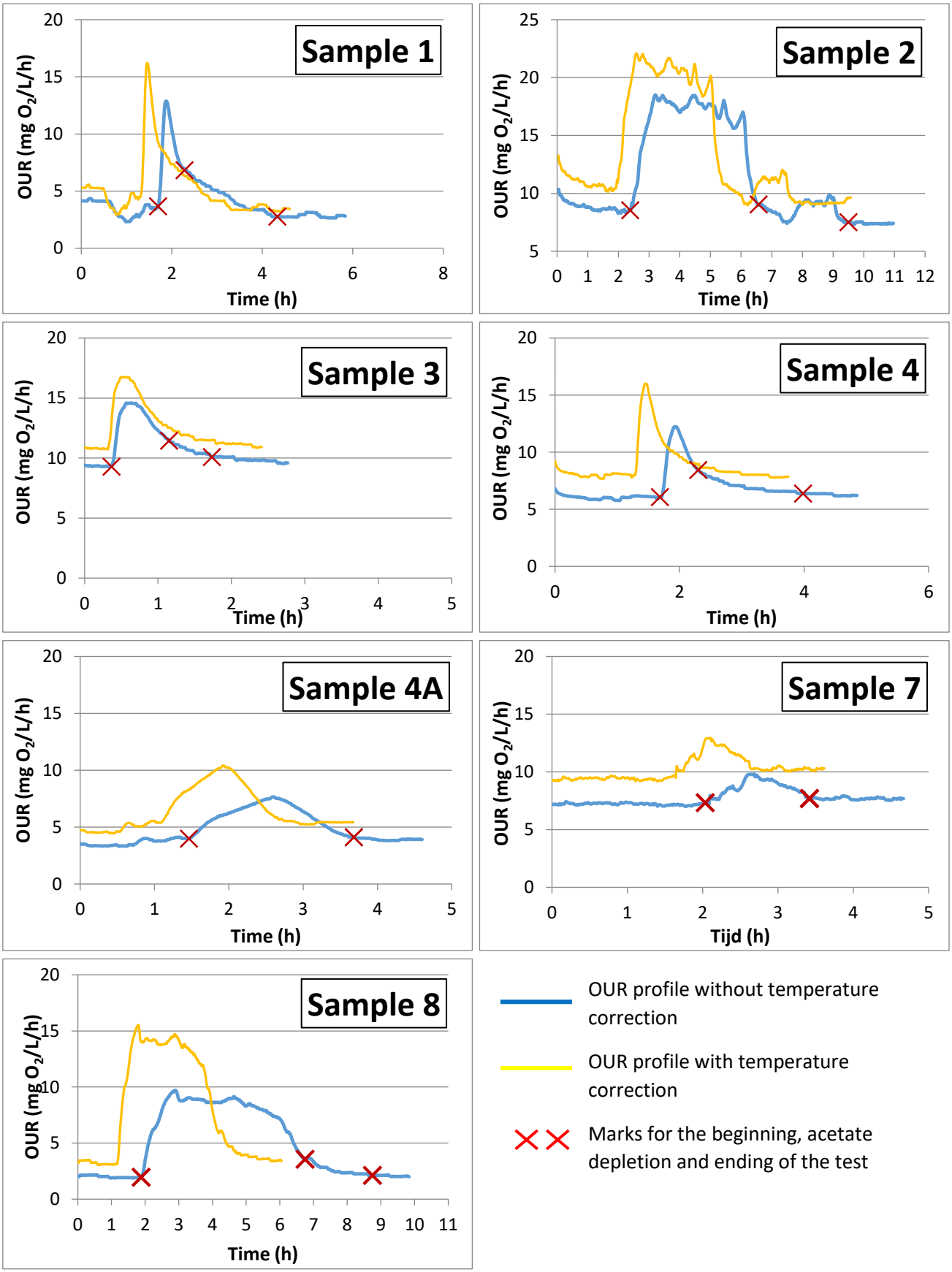

Figure 5: Obtained respirograms from different sample points

These results show higher values than indicated in the literature for both coefficients, e.g., for the SSAG model (Hoque et al., 2008), the ASM3 (Henze et al., 2000) or CWM1 (Langergraber et al., 2009). Also results from previous, similar researches show lower results (Ho, 2018; Pitzalis, 2018). However the higher yield values for Y_{STO} than Y_H are consequent with previous works.

Thus, the calculated growth coefficients for the soil based full-scale wetland show some similarities, but are more widely distributed, with higher values on average. The most plausible explanation for these high values would be the adsorption of substrate on the soil. This process, called biosorption, is a mechanisms were different components bind with the porous surface and cell structure of the biomass. By adsorbing, less oxygen is consumed which leads to higher yield values. This overestimation is consistent with the work of Ho (2018), where gravel was used instead of soil.

Furthermore variating results could be caused by an unequal distribution of aerobic and anaerobic/anoxic microorganisms in the soil. However, large errors could also occur due to the handled test method. Further investigation is needed to determine a proper standard method to measure microbiological activity with respirometric tests.

When using regression analysis to determine significant correlations between different parameters (p-value < 0.05), only a few significant correlations were observed. Within these correlations, very low R^2 values were observed (< 0.5). This indicates very weak relationships for the results in this research. Different correlations were observed between the temperature and the HRT, test duration or maximum OUR peak height. Between the level of endogenous respiration and the amount of VAS, HRT or duration of the test, and between the maximum OUR peak height with the concentration VAS or HRT. At last, small correlations were observed between Y_H and the tests duration, or between Y_{STO} and the HRT.

More clear relationships were expected, especially between the yield values and the amount of VAS, food-to-microorganisms ratio, temperature, etc. Nevertheless the amount of results is quite low to perform proper statistical analysis, so more samples with a larger number of tests are necessary for further investigation. The widely spread ranges of results could be due to several interferences, inaccuracies in the used methods or most important because of the soil composition. Therefore a better insight into the exact sample and biomass composition could give a better understanding of the biochemical processes and their results.

3.4 Respirogram profiles

Storage evidence was observed in 21 out of 25 results divided over 12x type I, 7x type II and 6x type III respirograms (figure 5). The presence of storage products confirms the SSAG model as more appropriate to describe the determination of stoichiometric parameters for aerobic substrate biodegradation. Comparable with previous researches, type I was the most common form and can be assumed to be the standard respirogram for microorganisms as the natural reaction mechanism for substrate biodegradation in normal circumstances, i.e., an intermittent feeding pattern in this case.

With this data, there is no direct link with the typical characteristics in order to obtain this type. Because of the typical slow rise of the OUR-profile, there can be assumed this is caused by a slow migration of substrate to the microorganisms. Or it could be an indication of a longer time needed for the microorganisms to adapt to new circumstances, i.e., aerobic conditions. In this way type II respirograms would occur as intermediate between the first and the third type.

At last, it could also be caused by microorganisms used to higher COD-concentrations and inconsistent loading rates. This would explain the absence of storage products and the slow response to new substrate addition. Further investigation is needed in order to determine the exact conditions to cause different respirogram types.

Type III respirograms were observed clearly at sample points where the highest loading rates take place in the wetland, i.e., the in- and outlet (table 1). However no evidence was found that higher growth yields automatically lead to type III respirograms.

4. Conclusions

The use of an LSF-respirometer in respirometric tests of soil filling media from a HSSF constructed wetland encountered several difficulties. Especially with the transposition to soil based samples. This was mainly remarkable at the respirometer box reactor. It was therefore adjusted. These difficulties resulted in a higher inaccuracy of the test results.

Notwithstanding the method has a high potential, further standardization is needed in order to design a proper method for the measurement of cell growth of microorganisms by aerobic biodegradation in soil media via LSF respirometers.

The used sampling method of soil samples worked properly.

Based on the HRT and VAS a specific preferential flow path is assumed mostly at the right-hand side,

around the clogged area at the centre of the bed. The HRT showed increasing results with the wetlands length, which is contrary to the expectations. The amount of VAS (i.e., biomass) decreases with the wetlands length, with maximums at sample point 2 and 4 and a minimum at the wetlands centre (point 5).

An average Y_H of 0.852 mg COD/mg COD was observed with a total range of 0.324 – 0.961 mg COD/mg COD. For the Y_{STO} the average is 0.895 mg COD/mg COD with a total range of 0.794 – 0.968 mg COD/mg COD. Higher values were observed according to the literature with a more spread range. However, the higher yield values for Y_{STO} than Y_H are consequent with previous works such as that of Ho (2018).

The clearly high yield values are most assumably due to substrate adsorption on the porous surface and cell structure of the biomass. This mechanism, called biosorption, will reduce the oxygen consumption which leads to an overestimation of the stoichiometric parameters. A method should be calibrated in order to inhibit this phenomenon or to develop a correction factor.

Few to no correlations were observed, with only weak relationships for a couple of paramaters. Nevertheless, the growth pattern of microorganisms throughout the wetland bed show significant differences. More tests are recommended in order to further investigate possible correlations (higher amount of tests = better statistic accuracy) and this would also help to test the methods' repeatability.

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