DETERMINING STOICHIOMETRIC PARAMETERS USING AN LSS RESPIROMETER FOR MATHEMATICAL MODELING OF CONSTRUCTED WETLANDS

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ABSTRACT

Despite being a natural treatment, constructed wetlands (from now on referred as CW) constitute an extremely complex system due to numerous physicochemical transformations that occur simultaneously. As a result, these solutions have been studied in greater detail using this model.

Most mathematical models currently available are based on proposed models for activated sludge (ASM) systems, which often comprise many parameters, both kinetic and stoichiometric. An example of this is CWM1- Constructed Wetland Model 1, one of the most popular models in CW. Due to a very small number of studies dedicated to determine model parameters for constructed wetlands, modeling studies for these systems often rely on the parameters obtained for activated sludge.

This study proposed to measure maximum heterotrophic growth yield (Y_H) of the biofilm from two constructed wetlands with subsurface flow using a LSS respirometer (Liquid-phase principle: Static gas, liquid Static). Tests were performed to biomass from two CW supplied with different organic load, however the study of CW feed with a lower load is in an early stage. Biofilm samples, used in respirometric tests, were obtained using 100 to 300 mL bulk volume of gravel and washing vigorously with 1.5 L of tap water. Tests had duration between 8 to 15 hours and sodium acetate was used as a soluble substrate.

The oxygen uptake curve, from the CW fed with higher organic loads, only revealed consumption of added substrate. The range of the stoichiometric Y_H was from 0.64 to 0.74 $gCQOgCQO^{-1}$ comprising the Y_H range of values for activated sludge and constructed wetlands with vertical flow.

Respirometric tests, carried out from CW biofilm fed with a lower load, revealed two distinct stages as observed in previous studies. First stage was associated with readily biodegradable substrate consumption and the second, associated with storage products consumption.

Keywords – constructed wetlands, respirometry, Y_H, oxygen uptake rate curve.

1 INTRODUCTION

Constructed wetlands are a sustainable solution for the biological treatment of effluents. This type of treatment takes advantage of natural degradation mechanisms in wetlands, for the pollutants removal on wastewater. The advantages of this type of solution include its optimum landscape integration and high sustainability, regarding reduced energy consumption and chemical reagents.

Despite being a natural treatment, built wetlands constitute an extremely complex system due to many physical and chemical transformations that occur simultaneously. Besides that, the increased knowledge about biological degradation mechanisms of wastewater treatment processes, resulted in the elaboration of several mathematical models, currently used as instruments for design, operation and optimization of treatment infrastructures. For that reason, constructed wetlands have been studied in greater detail with the use of modeling. Most of the currently available mathematical models are based on activated sludge (ASM) or wetland constructed models with parameters imported from activated sludge. It´s the case of CWM1- Constructed Wetland model 1 (Langergraber et al. 2009). This model comprises a high number of parameters, both kinetic and stoichiometric, deduced for activated sludge.

The obvious lack of specific information for this type of biological treatment indicates how important are developing studies, in way to obtain kinetic and stoichiometric parameters related to the constructed wetlands.

Currently, respirometric method represents an important support tool for modeling biological treatment systems, since it allows to obtain some necessary parameters for models calibration that could not be directly measured or determined by analytical methods. Respirometric consists in measuring the Dissolved Oxygen (DO) dynamics during substrate consumption by the biomass, resulting in an Oxygen Uptake Rate (OUR) curve. These curves allow the estimation of values for parameters related to the aerobic bioconversion of substrates and the identification of different consumption phases.

The present work aims to understand the development of biofilm from constructed wetlands using the respirometric method to determine the maximum heterotrophic growth yield (Y_H).

2 MATERIALS AND METHODS

2.1 Respirometer set-up

In this study, the respirometer used is an LSS type which works with a liquid-phase measurement principle and a static gas and static liquid operational regimen. It was developed at Instituto Superior Técnico.

Used respirometer includes a main reactor opened at the top but closed at the bottom, built from an acrylic cylinder. The main reactor has inlet-outlet ports for connection of the tubes. Respirometer also includes a reading cell, without aeration, made of glass. The system also includes an air compressor

(HAILEA V-20, China), a hydraulic pump (Flojet, China), a mechanical stirrer (BS VELP, Italy) and a dissolved oxygen sensor (ProODO, YSI, USA), which also gives temperature readings. The Figure 1, shows schematically all the elements that compose the respirometer system.

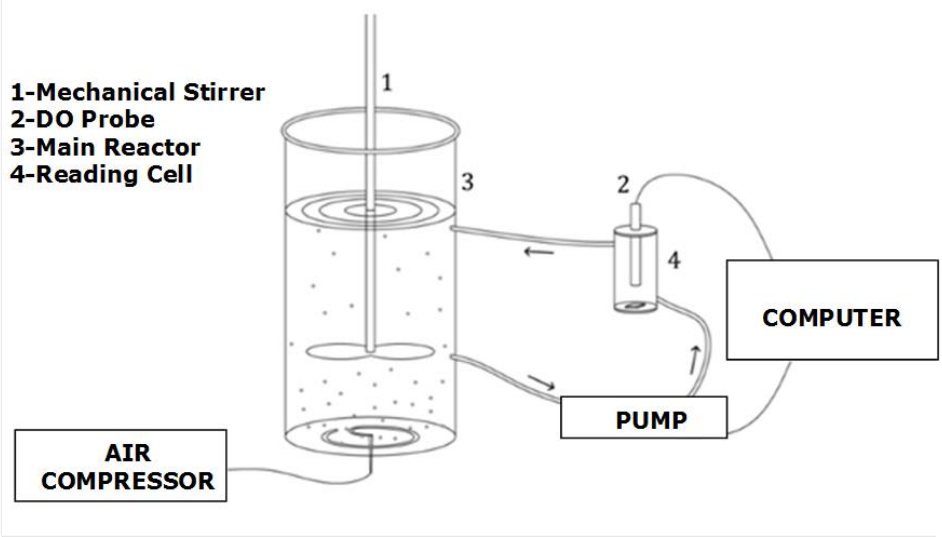

Figure 1 – LSS respirometer used during the experimental phase

Air compressor is connected through a rubber tube to the main reactor bottom, in order to provide necessary aeration to the suspended biomass.

In this respirometric system, liquid circulates between reactor and reading cell, where the probe is inserted. Connection between these two elements is made by a rubber and a cap, which prevents oxygen to be transferred between the cell and the outside.

DO sensor is connected to a computer, where readings are registered. The selected DO sensor uses a luminescence-based measurement principle, allowing less fouling problems.

Used pump, that makes liquid recirculate between reactor and reading cell, is also connected to a computer, allowing the control of pumping cycle times. For this experiment, OUR tests were conducted with a cycle, including a 3-minute idle period with no recirculation (static liquid) and a 2-minute pumping period. Since, during the idle period, there is no renewal of dissolved oxygen, the decrease of dissolved oxygen results only from consumption by the biomass.

Mechanical stirrer is used to promote a continuous biomass suspension in the main reactor, while air compressor aeration keeps dissolved oxygen levels close to saturation, so that DO is not a limiting factor in the OUR measurement.

2.2 Biofilm source and sampling

This study was carried out with biofilm samples, taken from two horizontal subsurface flow, constructed wetland installed in Hydraulics Laboratory of Instituto Superior Técnico, in Lisbon,

Portugal. Both constructed wetlands were planted with *Scirpus holoschoenus*, on a gravel media with a porosity of 30%. The main difference between constructed wetland 2 (CW 2) and constructed wetland 9 (CW 9) is the way that they are fed. The CW 2 is fed with synthetic sewage with a Chemical Oxygen Demand (COD) content of 3838 mgO_2L^{-1}. Feeding was done at a rate of 10 L per day, five days a week, with a rest period during weekends. The CW 9 was fed 5 times a week with quantities much lower than that CW 2, resulting from diluted leftovers from synthetic sewage. Although, it was not quantified in precise terms the COD, is estimated that the mass load is approximately 10 gO_2 / m² day.

To obtain each biofilm sample, a volume between 100 and 300 mL of gravel was carefully removed from constructed wetland center. In order to detach the biofilm, 1,5 L of tap water at room temperature was added to the gravel sample followed by a vigorous manual shaking period, after which most of the biofilm was observed to be in suspension.

Finally this volume is placed in the main reactor by adding tap water to a final volume of approximately 2.5L.

2.3 Respirometric test

Respirometric test allows to determine the OUR curve corresponding to the oxidation of a specific amount of rapidly biodegradable substrate. The OUR curve obtained corresponds to the line that best fits the values of DO concentration, and which minimizes distances of measured values along time, in idle periods.

After placing the sample in main reactor, the biofilm suspension was aerated until endogenous respiration level was attained. This procedure ensures that oxygen consumption, during the test, was due only to the oxidation of the added substrate.

Once endogenous respiration level was attained, were added 200mL of a sodium acetate solution corresponding to a carbonaceous substrate, which will allow measuring the oxygen consumption capacity of biofilm. Acetate concentration, in the added solution, was adjusted in order to give COD levels in reactor, between 83 and 519 mgL^{-1}. This values range is based on previous studies, where several concentrations of acetate were tested inside the reactor. COD content acetate solution was measured using cuvette tests (25-1500 mg/L,MERCK).

Respirometric ends when endogenous respiration is again attained. Depending on the amount of substrate added, the tests had durations between 8 and 15 hours.

In order to avoid interference from nitrification, a nitrification inhibitor (NTH 166 600, WTW) was added to biomass suspension at the beginning of each respirometric test.

2.4 Calculations for Y_S e Y_{STO}

In real waste water treatment, biofilm is exposed to variable concentrations of different substrates. It means that microorganisms experienced two contrasting situations: feast conditions, when external

substrate is available in excess, and famine conditions, in the absence of external substrate.
In order to assure biomass response on this variability, Gujer et al. (1999) proposed the ASM3 model that introduces a phenomenon of carbon storage sources, observed by several researchers. This model assumes that substrate is first stored before being used for biofilm growth. However, experimental data have demonstrated the existence of simultaneous storage and growth processes during feast conditions. In the famine phase, microorganisms consume previously created storage products, which function as a sort of reserve. Based on this, the SSAG (Simultaneous Storage And Growth) model, proposed as a complement to the ASM3 model, (Hoque et al. 2010).

In this study, identifying the period of readily biodegradable organic matter (S_s) consumption and the period of storage products consumption (that ends when OUR settles back at endogenous level), was performed visually, following methodology proposed by Andreottola et al. (2007) and Ortigara et al. (2011), as represented in Figure 2, which is a graphical representation of DO consumption profiles associated with these two phases: the first one corresponds to a period in which the rapidly biodegradable substrate is consumed, and the second to a period in which the storage products are consumed.

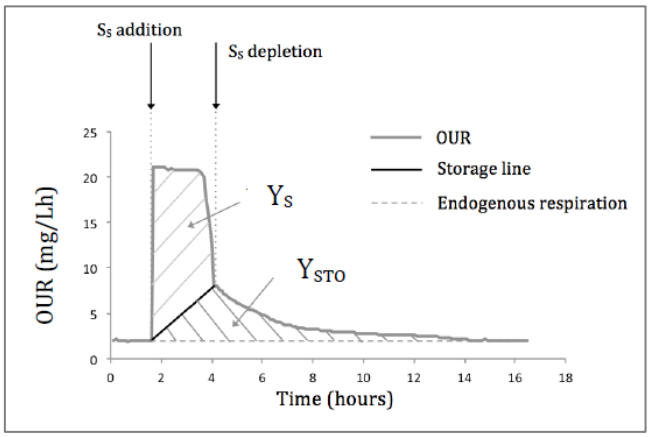

Figure 2 – Graphical visualization of used method to distinguish between initial (YS) and storage (YSTO) yield values

Besides that, Figure 2 also indicates the calculation procedure for two yield values, according to a simplified method proposed by Ortigara et al. (2011). The initial growth yield value (Y_S) was obtained using equation 1 below.

$$Y_S = 1 - \frac{\Delta O_{2\ acetate}}{S_S} = 1 - \frac{\int_{t_0}^{t_f} [OUR(t) - OUR_{endogenous}(t) - OUR_{STO}(t)]dt}{S_S} \qquad \text{(eq.1)}$$

Where S_S is the initial concentration of added substrate (acetate - COD, mgO_2L^{-1}), $\Delta O_{2\ acetate}$ will be the amount of oxygen consumed for the oxidation of added substrate, OUR (t) is the oxygen consumption rate at the end of the test, $OUR_{endogenous}(t)$ is the endogenous respiration level, OUR_{STO} is the oxygen consumption rate at the beginning of famine phase, t_0 is the instant substrate is added and t_f is the instant that all the added substrate has been consumed.

The storage yield was then calculated according to equation 2.

$$Y_{STO} = 1 - \frac{\Delta O_{2\ STO}}{S_S} \quad \text{(eq.2)}$$

Where S_S is the initial concentration of added substrate (acetate - CDO, mgO_2L^{-1}) and $\Delta O_{2\ STO}$ is the oxygen consumption associated to storage

The growth yield coefficient, Y_H refers to the total growth of the heterotrophic biomass by aerobic conditions. If the OUR curve do not consumption of stored products, Y_H is equal to Y_S.

2.5 Temperature corretion

Temperature is a parameter with a strong influence on microorganism's metabolism, affecting organic matter's oxidation rate. In general, higher temperatures accelerate the biological decomposition reactions of organic matters, thus influencing its removal efficiency by the organisms.

Because respirometry interprets and evaluates the metabolism of microorganisms, OUR curves, obtained at room temperature, are affected by daily temperature variations. As such, it isn't easy to accurately identify endogenous respiration. In order to eliminate the influence of temperature variations, OUR curve was corrected by adopting a reference temperature of 20 °C.

So, in respirometry tests performed, temperature in the reading cell was continuously registered and its correction was performed using equation 3 indicated in Ortigara et al. (2011).

$$OUR_{20°C} = \frac{OUR_T}{\alpha^{(T-20°C)}} \ (\alpha = 1.08) \ (3)$$

Where $OUR_{20°C}$ is the oxygen consumption rate corrected to the temperature of 20˚C (mgO_2/Lh), OUR_T is the oxygen consumption rate at a temperature T (mgO_2/Lh), T is the temperature measured at each value of OUR (˚C) and α is the Arrhenius constant.

3 Results and Discussion

Respirometry tests results, performed in CW2, revealed an OUR profile with only one phase related to the external substrate consumption. After acetate addition, a high OUR peak can be identified. After this initial high value, OUR decreases down to the endogenous level. During this decreasing period, OUR curve shows only one slope. Note that the endogenous OUR level increases from its initial value. That result probably occurs from biomass growth.

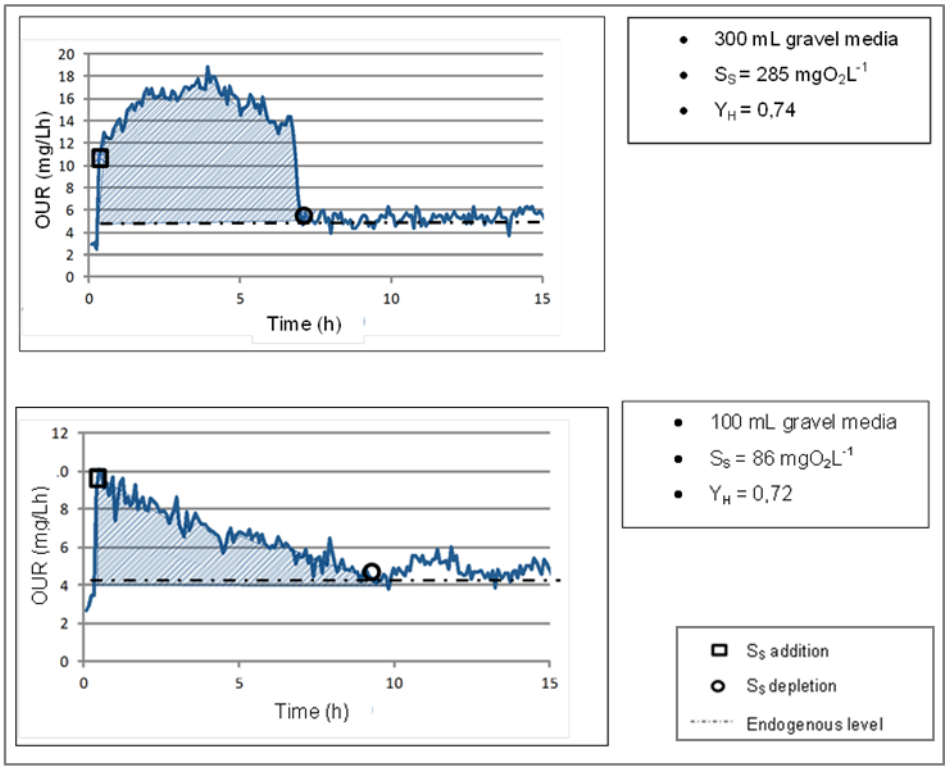

Figure 3 – OUR profile obtained for CW2

Biofilm from CW2 was already studied in the past, so, this study made a comparison with the previous one. In 2014, CW were fed with a COD content of 757 mgO_2L^{-1}. As Figure 3 reveals, OUR profile no longer presents two phases as it was in the past. Comparing with carried out tests in the same CW, the most significant change is the absence of evidence of storage. This difference in behavior is mainly due to the increase in the amount of organic load of the influent solution with which the CW was fed.

In 2014, when the CW was fed with a lower amount of organic matter, the available substrate amount limited the growth of biofilm, in some periods. As such, microorganisms capable of producing and storing polymers had a competitive advantage, compared to microorganisms without such ability, and tests performed revealed the existence of storage phenomenon. This mechanism allowed them to survive after food exhaustion. However, when CW was fed with an amount of organic matter about five times higher (January 2016), storage was no longer a fundamental mechanism for microorganism's survival. Although biofilm was also subject to dynamic conditions of substrate availability, the duration of absence periods was lower, considering higher feed concentration in the medium culture. The concentration change of the affluent solution probably favored the microorganisms with direct substrate use to the detriment of storage solutions, influencing the biodegradation process of organic matter.

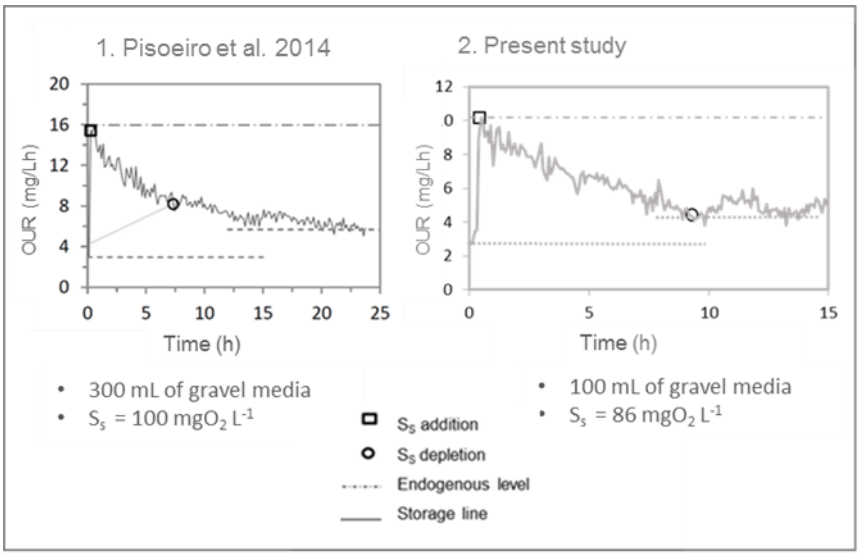

Figure 4 – Comparison with respirometric test performed in the past

Respirometry results tests, performed in CW9, revealed an OUR profile with two different phases (Figure 6). The first one is related to the external substrate consumption (feast conditions), while the second one corresponds to the consumption of the previously stored products (famine conditions). After acetate addition, a high OUR peak can be identified. After reaching the maximum level, OUR drops to a level higher than the endogenous respiration, followed by a gradual decrease down to endogenous level. During this decreasing period, two slopes can be identified: an initial higher slope, related to external substrate consumption, and a second lower slope, related to the previously stored products consumption.

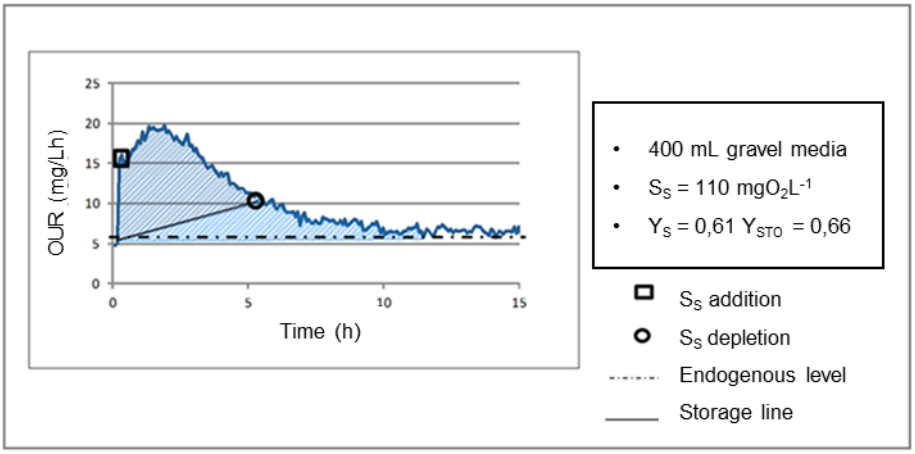

Figure 5 – OUR profile obtained for CW9

4 Conclusions

The respirometric tests performed with biofilm from constructed wetlands feed with higher organic load (3838 $mgO_2\ L^{-1}$), didn't showed evidence consumption of the previously stored products but only direct consumption of the external substrate.

However, constructed wetlands fed with lower organic load, presented two distinct phases. The first is related to the external substrate consumption, while the second one corresponds to the consumption of the products previously stored.

The ranges of values obtained for Y_S and Y_{STO} comprise the range of values found in literature for proposed models of activated sludge systems as well as the value obtained by Ortigara et al. (2011) for a vertical flow constructed wetland.

It was also found that biofilm sample, required for a respirometric test, had a lower volume in the case of constructed wetlands, fed with higher organic load. This fact is probably due to a lower development of the biofilm in constructed wetlands fed with less organic load, resulting from the less availability of substrate. In this way, the importance of properly determining the substrate / biomass ratio in each CW was identified because this will allow a better understanding of the development of the biofilm through the stoichiometric coefficients.

This study will continue to determine the transition conditions between the two types of observed behavior, namely the direct consumption of substrate and the consumption of substrate followed by consumption of storage products.

The results obtained in this study are relevant for application in several studies, namely modeling, since they allow a better understanding of the systems under study, they also make possible the optimization of their treatment efficiency.

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