"Constructed Wetlands: Comparison of the latest mathematical models and interpretation of respirometric data"

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1 INTRODUCTION

The Wetlands are naturally formed areas where the proliferation of bacteria is guaranteed by the excellent conditions for the survival of these organisms. Their nature depends on the type of vegetation present, which directly influences the growth of a certain type of bacteria: this is why it speaks of Phyto-purification.

The possibility to study and controls the biological phenomena that occur within the Wetlands encourages researchers to invest resources. The main use that derives from this is explained by the ability of these organisms to biodegrade any type of wastewater, both industrial and civil and agricultural. We are therefore talking about Constructed Wetland.

One of the main problems of this research topic is precisely the mathematical modeling of the removal processes that occur within the CW.

The purpose of this thesis, therefore, is the calibration of the parameters of a mathematical model to describe the processes of oxygen removal, within the CW, by means of respirometric tests conducted in the laboratory of environment of the IST, University of Lisbon, under the supervision of Professor Ana Galvão and PhD student Joana Pioeiro.

After the first period spent in the laboratory acquiring respirometric data, the attention was shifted to the mathematical model chosen. This model is a rectification of the ASM 3 model, mainly used for activated sludge, with some differences in the processes involved.

The calibration work, as shown in chapters 7 and 8, has brought reliable results by providing researchers with possible values assignable to the model parameters which describe the system consistently with what was hypothesized and what was expected.
2 CONSTRUCTED WETLANDS AND WASTEWATER TREATMENTS

Treatment Wetlands (TW) are the new frontier of the technology in biological wastewater treatments.

They are engineering systems designed to optimize and to improve natural processes of wastewater compound biodegradation; this is the reason why Treatment Wetlands are recognized environmentally friendly and sustainable, with low impact.

Treatment Wetlands have many advantages, the most relevant are surely low maintenance costs and durability (this type of treatments are able to guarantee their performance over time thanks to low dependence on input variations).

All types of wastewaters are possible to treat: domestic and industrial sewage and also agricultural polluted water.

Treatment Wetlands can be divided in two types with reference to water flow:

a) Subsurface Water Flow; further classified in Horizontal Flow (HF), Vertical Flow (VF), French Vertical Flow (FVF)

b) Free Water Flow
Bacteria and biofilm grow during the process increasing their volume, this phenomenon is called Clogging in which the porous media is obstructed by the new mass created. For this reason Treatment Wetlands are used for secondary treatment of wastewater.

**Vertical Flow Wetlands** are applied as French Vertical Flow Wetland providing the possibility of unique process in order to avoid the primary treatment and, indirectly, also Clogging phenomenon. This applied version of VF wetland allows good efficiency of the system with less costs in terms of time and money due to the primary treatment.
Free Water Flow Wetlands, instead, are different from the previous because water flows above the media bed. These types are generally used as tertiary treatments when it is required to remove a specific pollutant that was not sufficiently removed in the secondary treatment.

Each CWs have four stages into occur different removal levels. The first three (Preliminary, primary and secondary) are the main stages with more relevant impact on removal process, a tertiary stage could be introduced if it is necessary, for example in case which a pollutant has not been removed in the previews stages (Sperling & Chericharo, 2005).
<table>
<thead>
<tr>
<th>Stages</th>
<th>Removal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preliminary</td>
<td>• Coarse suspended Solids (larger material and sand)</td>
</tr>
<tr>
<td>Primary</td>
<td>• Settleable suspended solids</td>
</tr>
<tr>
<td></td>
<td>• Particulate BOD (associated to the organic matter component of the Settleable suspended solids)</td>
</tr>
<tr>
<td>Secondary</td>
<td>• Particulate BOD (associated to the particulate organic matter present in the raw sewage, or to the non-settleable particulate organic matter, not removed in the possibly existing primary treatment)</td>
</tr>
<tr>
<td></td>
<td>• Soluble BOD (associated to the organic matter in the form of dissolved solid)</td>
</tr>
<tr>
<td>Tertiary</td>
<td>• Nutrients</td>
</tr>
<tr>
<td></td>
<td>• Pathogenic organism</td>
</tr>
<tr>
<td></td>
<td>• Non-biodegradable compounds</td>
</tr>
<tr>
<td></td>
<td>• Metals</td>
</tr>
<tr>
<td></td>
<td>• Inorganic dissolved solids</td>
</tr>
<tr>
<td></td>
<td>• Remaining suspended solids</td>
</tr>
</tbody>
</table>

Tab 2.1 Removal processes in each treatment steps

Each treatment levels have a specific role in the removal efficiency of the system:

1. **Preliminary treatment** is the removal of coarse solids.
2. **Primary treatment** has the objective of removing settleable organic matter.
3. **Secondary treatment** has the aim to remove the organic matter and possibly nutrients (Nitrogen and Phosphorous) by biological mechanisms.
4. **Tertiary treatment** considers the removal of specific pollutants remaining after the secondary treatment.
2.1 Removal Efficiency

All these stages and the whole process are subordinate to the main applicable problem: Removal Efficiency on each level.

Commonly used formula by the researchers is the follows:

\[ E = \frac{C_0 - C_e}{C_0} \cdot 100 \]

Where:

- \( E \) = Removal Efficiency (%)
- \( C_0 \) = influent concentration of the pollutant (mg/L)
- \( C_e \) = effluent concentration of the pollutant (mg/L)

2.2 Application Field

Between FWS CWs and SSF CWs, FWS CWs are more efficient at removing organic matter and suspended solids than at removing nitrogen and phosphorus compounds.

However, their performance is highly dependent on climatic and vegetation conditions.

SSF CWs, on the other hand, are more efficient at removing organic substances, suspended solids, pollutants and heavy metals and are also less sensitive to low temperatures.

As a result of Clogging, SSF CWs have a shorter lifetime than FWS CWs, which can guarantee more than 10 years of service.

The combination of CWs with other processes, such as for examples algae control or the reuse of irrigation water, provides an increasing of the efficiency thanks to the nitrogen, phosphorus and organics removal power of these biological treatment.
The CWs are widely used in treatment of domestic and municipal wastewater, but recently have been carried out, by some researchers, studies concerning the possibility to use CWs also to treat many different types of wastewater from industries or agricultural sewage.

Although there are many advantages choosing CWs, this type of treatment plant is subordinate to the environmental condition of the land where to install it. The most limiting factor for their application are surely the land requirements needed for their good working conditions. Places with high population density and scarce resources don’t respect those requirements.

In addition, in order to achieve higher removal performance, artificial aeration could be introduced in the project, this increase the lifecycle costs of CWs.

2.3 Plants role in CWs

The main-role in WWTP CWs is played by plants, which have the responsibility to provide and maintain optimal environmental conditions.

Not all plant species can be used. For correct and sustainable design of CWs, plants selected should have tolerance of waterlogged-anoxic and hyper-eutrophic besides adaption to extreme climates.

Globally, more than 150 macrophyte species have been used in CWs, including emergent plants, submerged plants, floating leaved plants and free-floating plants. Despite the high number mentioned before, just few of them are often planted in CWs; some of them are following reported:

- Phragmites spp. (Poaceae)
- Typha spp. (Typhaceae)
- Scirpus spp. (Cyperaceae)
- Iris spp. (Iridaceae)
- Juncus spp. (Juncaceae)
- Eleocharis spp. (Spikerush)

The most frequently used *submerged plants* are:

- Hydrilla verticillata
- Ceratophyllum demersum
- Vallisneria natans
- Myriophyllum verticillatum
- Potamogeton crispus

The *floating leaved plants* are mainly:

- Nymphaea tetragona
- Nymphoides peltate
- Trapa bispinosa
- Marsilea quadrifolia

The *free-floating plants* are:

- Eichhornia crassipes
- Salvinia natans
- Hydrocharis dubia
- Lemna minor

### 2.4 Types of wastewater systems

There are many different types of biological wastewater treatment systems such as:

1. *Stabilization Ponds*, wastewater remains in the ponds for many days. Bacteria grow in the liquid phase, particulates in suspension tends to settle at the
bottom of the ponds. Oxygen required by aerobic bacteria is provided by algae through photosynthesis. High land requirements.

2. **Land disposal**, for wastewater treatments or water reuse or landscape irrigation. Wastewater is applied directly to the soil providing nutrients and water for plants growth. CWs belong to land disposal treatments.

3. **Anaerobic system**, oxygen is converted anaerobically by bacteria in the reactor.

4. **Activated sludge**, high removal power of the system due to the high concentration of bacteria during the process. Recirculation of the settled solids in a second sedimentation tank allows, along with mechanical aeration, high biomass concentration in the system corresponding to high removal efficiency. Attention to sludge in excess that needs to be removed and stabilized.

![Fig. 2.4 Photo of an Activated Sludge System](image)

### 2.5 Substrate in Constructed Wetlands

Substrate is a fundamental design parameter in CWs and SSF CWs, because it can provide a comfortable growing medium for plants and allow wastewater flow.
Substrate has also an important role in absorbing various pollutants such as phosphorus. Selection of best substrates to use in CWs for industrial wastewater treatment is a crucial issue.

The selection of substrates is determined by the “hydraulic permeability” and the “capacity of absorbing pollutants”. Low hydraulic conductivity could create perfect condition for Clogging, decreasing the system efficiency. Low adsorption capacity could affect the long-term removal performance of CWs.

Several studies were carried out with the purpose to find optimal substrate for CWs; most frequently used substrates mainly include natural material, artificial media and industrial by-product, such as gravel, sand, clay, calcite, marble, vermiculite, slag, fly ash, bentonite, dolomite, limestone, shell, zeolite, wollastonite, activated carbon, light weight aggregates. From these studies, results showed that substrates such as sand, gravel, and rock have negative influence on long-term phosphorus storage, instead those products with high hydraulic conductivity and phosphorus sorption capacity could be good candidate to be used in CWs. Other studies also provided some information on substrate selection in order to optimize the removal of nitrogen and organics, on this purpose have been introduced substrates such as alum sludge, peat, maerl, compost and rice husk (Wu, et al., 2014).

2.6 Hydraulic of Constructed Wetlands

One of the first CWs controlling factors is the Hydraulic of the system, regulating the flow rate is possible to achieve a satisfactory treatment performance. The optimal design of hydraulic loading rate (HLR) and hydraulic retention time (HRT) improve the removal efficiency of CWs. Greater HLR promotes faster flow of wastewater through the media, thus reducing the optimum contact time. On the contrary, using an adequate flow rate, microbial community have enough time to be established in CWs guaranteeing the appropriate contact time to remove contaminants at a longer HRT.
Huang et al. (2000) concluded that ammonium and TN concentrations in treated wastewater decreased drastically with increasing HRT in CWs treating domestic sewage. Similarly, Toet et al. (2005) found positive nitrogen removal in CWs with HRT of 0.8 days comparing with the results with 0.3 days residence time.

A low HRT in CWs may be associated with incomplete denitrification and it is reported that nitrogen removal requires a longer HRT compared with HRT necessary for organics removal. In addition, effect of HRT depends on the dominant plant species and temperature, along with those factors influencing the hydraulic efficiency of wetlands.

Despite what written before, some researchers reported a long-term evaluation of fully matured VF CWs for treating synthetic wastewater and showed that the wetland systems reached higher nitrogen and organics removal as the HLR increased.

### 2.7 Constructed Wetlands Design Criteria

As explained in precedent paragraphs, many different parameters are involved in a correct design of a Constructed Wetland system. These treatment technologies are based on biological processes which are not so easy to control, they need a guide to be designed reporting at least recommendations for a good realizing project. The following table represent an example:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Design criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bed size (m²)</td>
<td>Larger if available</td>
</tr>
<tr>
<td>Length to width ratio</td>
<td>3.1–5.1</td>
</tr>
<tr>
<td>Water depth (m)</td>
<td>0.3–0.5</td>
</tr>
<tr>
<td>Hydraulic slope (%)</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>Hydraulic loading rate (m/day)</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Hydraulic retention time (day)</td>
<td>5–30</td>
</tr>
<tr>
<td>Media</td>
<td>Natural media and industrial by-product preferred, porosity 0.3–0.5, particle size &lt;20 mm (50–200 mm inflow and outflow)</td>
</tr>
<tr>
<td>Vegetation</td>
<td>Native species preferred, plant density 80% coverage</td>
</tr>
</tbody>
</table>

*Tab. 2.2 Recommendations on the design and operation of CWs for wastewater treatment (Wu, et al., 2014).*
In the last years researchers leaded their work to increase the knowledge concerning design and operation of CWs obtaining great results in terms of contaminant removal efficiencies and sustainable applications of this treatment system. However, CWs still have some limitations and further research needs to be done.

Fig. 2.5 shows the improvement and future development in this way.

![Diagram showing sustainability of CWs]

*Fig. 2.5 Current developments and future considerations to improve the sustainability of CWs (Wu, et al., 2014).*
3 Classification of Wetlands

What are Constructed Wetlands?

In general, Wetlands are a transition zone where occurs saturated conditions between water and soil. They create an ecosystem with a specific hydrology and hydraulic providing perfect environmental conditions to the formation of a unique vegetation species.

There are two main types of Wetlands: Natural and Constructed.

The difference between them is principally that the first one is part of the land which achieve wetland condition without anthropogenic influence; instead, the second one are engineering system designed to optimization of the physical, chemical and biological processes in order to remove pollutants from the water, in other word is an improvement of natural wetland (Fonder & Headley, 2010).

In order to guarantee an efficient removal process, treatment wetlands can be used as a complex wastewater treatment system with different set of pollutant and pathogen removal pathways.

Usually, typical wastewater treatments are designed with specific removal purpose. More unit operations are involved in the process to optimize the efficiency, thus multiple removal pathways occur simultaneously in different reactors.

Wetland plants play several important roles in treatment wetlands. Primarily, their roots and rhizomes provide attachment sites for microbial biofilms increasing the biological activity per unit area compared to open water systems such as ponds.

Another important goal of plants is to distribute the flow avoiding water short-circuiting, this has an important objective to bring oxygen through the porous media fueling aerobic and anoxic microbial processes.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Main Removal Mechanisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suspended Solids</td>
<td>Sedimentation and filtration</td>
</tr>
<tr>
<td>Organic matter</td>
<td>Sedimentation and filtration for the removal of particulate organic matter, biological degradation (aerobic and/or anaerobic) for the removal of dissolved organic matter</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>Ammonification and subsequent nitrification and denitrification, plant uptake and export through biomass harvesting</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>Adsorption-precipitation reactions driven by filter media properties, plant uptake and export through biomass harvesting</td>
</tr>
<tr>
<td>Pathogens</td>
<td>Sedimentation, filtration, natural die-off, predation (carried out by protozoa and metazoa)</td>
</tr>
</tbody>
</table>

*Tab 3.1: Main mechanisms for pollutant and pathogen removal in treatment wetlands.*

**Organic Matter**

Organic matter is the input of the system from an engineering point of view. It can be classified in many ways as particulate and soluble organic matter, each one associated to a different removal mechanism which depends on treatment wetland design. Chemical and Biochemical Oxygen Demand are both used to measure organic matter.

As described in the previous chapter, wetlands can be divided in two categories: surface flow and subsurface flow. The main difference is the flow system due to the porous conditions of the media.

Fonder and Headley proposed in 2010 a tree chart, reported below, which shows wetlands classification:
Fig 3.1 Tree chart for wetland classification by (Fonder & Headley, 2010)
3.1 Horizontal Subsurface Flow Wetlands

The origins of this type of configuration are in Germany in 1960s, common abbreviation of Horizontal Subsurface flow is HSSF which many publications refer to.

As presented in the (Dotro, Günter, Molle, Nivala, & Puigagut, 2017) common scheme of this type of CW is presented in the figure below:

![Fig 3.2 Schematic representation a typical horizontal subsurface flow](image)

The saturated condition of the gravel bed are one of the most important point of HSSF which, together with emergent wetland plant types, represent a typical configuration.

As shown in Figure 3.2, water enters in the gravel media and flows through the system.

All system needs to be isolated from the rest of the soil to prevent a contamination in the processes which occur in the media. To this purpose the whole bed is
surrounded by a geotextile membrane with plastic matrix to impermeabilize the interface between the Wetland and the rest of the soil.

Figure 3.3 shows the secondary treatment applied to the effluents of primary treatment; figure below instead shows the tertiary treatment also designed as HSSF system:

“For secondary treatment of domestic wastewater, the gravel depth is generally 0.5 to 0.7 m and the water level is kept 5 – 10 cm below the surface. In tertiary treatment applications in the UK, the depth of the basin is 1.0 to 1.5 m, of which approximately 0.60 m filled with gravel. HF systems in the UK are generally made with a longitudinal sloped (1%) to facilitate draining of the bed. The remaining bed volume is used for water storage during high flows or storm events” (Dotro, Günter, Molle, Nivala, & Puigagut, 2017).
<table>
<thead>
<tr>
<th>Treatment Step</th>
<th>SPAIN</th>
<th>US</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific surface area requirement (m²/PE)</td>
<td>10</td>
<td>5 – 10</td>
</tr>
<tr>
<td>Maximum areal organic loading rate (g BOD₅/m²·d)</td>
<td>6</td>
<td>4 – 8</td>
</tr>
<tr>
<td>Hydraulic loading rate (mm/d)</td>
<td>20</td>
<td>20 – 40</td>
</tr>
<tr>
<td>Gravel size (mm)</td>
<td>5 – 6</td>
<td>&gt; 4</td>
</tr>
<tr>
<td>Distribution system</td>
<td>Subsurface pipes</td>
<td>Subsurface pipes</td>
</tr>
</tbody>
</table>

Tab 3.2 Main design parameters chosen by different researchers in Spain and US

3.2 VERTICAL FLOW WETLANDS

Vertical Flow Wetland are typically utilized to remove ammonia nitrogen from wastewater, in particular they are implemented as secondary treatment in domestic wastewater treatment plants processes.

This type of wetlands are very versatile, this particularity allow to create many variations of Wetlands combining different wetlands type, in order to construct an improved and optimized pollutant removing system, for examples French VF (Dotro, Günter, Molle, Nivala, & Puigagut, 2017).

One of the first VF Wetlands system was developed by (Greiner & Jong, 1984); the result of primary treatment was the inlet of the system and immediately filtered by
the porous media composed by sand and gravel bed. The loading method of inlet was intermittently in order to guarantee a homogenous settling distribution.

Figure below shows a scheme of typical Vertical Flow Wetland composed by sand and gravel on the bed and emergent plants on the surface.

VF Wetland system has a strong ammonia removal power, it is in grade to oxidize high amount of ammonia in domestic wastewater.

Such an important characteristic is due to the nitrifying capacity.

It’s necessary to pay specific attention to the hydraulic design system; VF Wetlands doesn’t work under saturated condition (as Horizontal Flow Wetlands), the inlet flow is intermittent and some design specifications applied in HF wetlands are not applicable in VF.
As mentioned before, VF Wetlands has strong removal capacity which permit a good removal efficiency of organic matter and nitrogen ammonia during aerobic microbial processes.

Solids and pathogenic organism, instead, are treated with a physical filtration.

In the following Figure 3.5 are given some design guidelines for the thickness of different soil layer:

![Diagram of VF Wetlands with design guidelines for soil layer thickness](image)

<table>
<thead>
<tr>
<th>Layer</th>
<th>European type</th>
<th>French type</th>
<th>American type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sand ((d_1))</td>
<td>10 cm</td>
<td>0 cm</td>
<td>30 cm</td>
</tr>
<tr>
<td>Fine gravel ((d_2))</td>
<td>15 cm</td>
<td>50 cm</td>
<td>10 cm</td>
</tr>
<tr>
<td>Medium gravel ((d_3))</td>
<td>10 cm</td>
<td>20 cm</td>
<td>25 cm</td>
</tr>
<tr>
<td>Cobbles ((d_4))</td>
<td>15 cm</td>
<td>20 cm</td>
<td>15 cm</td>
</tr>
<tr>
<td>Total (d)</td>
<td>50 cm</td>
<td>90 cm</td>
<td>80 cm</td>
</tr>
</tbody>
</table>
The flow which pass from fine gravel to the drainage layer need to across an intermediate layer to prevent grains.

Coarse gravel at the bottom layers provide perfect drainage conditions along with drainage pipes. Main scope is to bring oxygen to the deepest layer guaranteeing good aeration condition.

Another aspect, extremely important from a practical point of view, is to prevent the grain migration to the layers below, otherwise the efficient of the system is compromised. In order to avoid this phenomenon, Terzaghi Rule is adopted (as suggested in (Sherard, 1984)):

\[ \frac{D_{15}}{d_{85}} \leq 4 \]

Where:
- \( D \) = grain size of the transition layer
- \( d \) = grain size of the main layer

### 3.3 Hybrid System

The different types of CWs described above can be combined together to create new systems called "hybrids".

Their creation is often necessary to overcome the impossibility of using a simple Constructed Wetland or to improve the performance of pollutant removal.

Among the combinations we can mention HSSF Ws in series or HSSF W with the addition of some parts of VF Ws.
Their application in Europe has been very successful mainly because of the strict rules of removal of ammonia from wastewater and these CWs ensure efficient operation from this point of view.

These denitrification processes take place in a bed of VF where the ammonia is transformed into nitrate and the solid and organic matters are filtered, the denitrified product is then directed to a HSSF bed type for the total removal of nitrogen.

The most diffused example of Hybrid system is French Vertical Flow Wetland.

An important aspect of F VF Wetland is that the simplicity.

There are no primary treatments or septic tanks, the biological treatment remains unique. In the design phase, an important practical detail should also be considered: under the action of the wind, plants tend to bend, preventing the infiltration of water into the ground; to avoid this phenomenon, a network of small openings in the ground is created to facilitate infiltration.

![Scheme of typical French Vertical Flow Wetland](Dotro, Günter, Molle, Nivala, & Puigagut, 2017)

Fig 3.5 Scheme of typical French Vertical Flow Wetland (Dotro, Günter, Molle, Nivala, & Puigagut, 2017)
3.4 Free Water Surface Wetlands

In nature, the Wetlands are found almost exclusively in the form of Free Water Surface because they have a purely superficial fluid flow, often there is no easily permeable soil especially in areas where the Wetlands occur most frequently.

Depending on the nature of the FWS CW, different structures may occur:

- Coated and uncoated
- Variable or constant depth
- Partially or completely vegetated
- Submerged, floating or emerging vegetation

The nature of the FWS Wetlands does not allow a total infiltration of the fluid so the flow will be purely horizontal (surface). The soil is composed of a granular matrix at the base with the function of supporting the Wetland, while the surface has impermeable characteristics and a type of vegetation able to survive even long periods of complete immersion. The speed of the flow is very low for this reason it is suitable for tertiary treatments. Other uses of the FWS Wetlands may be "floating treatments" such as rainwater purification.

![Fig. 3.6 Scheme of Free Water Surface Constructed Wetland (Dotro, Günter, Molle, Nivala, & Puigagut, 2017).](image-url)
Fig. 3.7 Main Processes in FWS CWs, (Wallace & Knight, 2006).
In this chapter are reported the descriptions of every parameters involved in wetland processes.

In particular, have been given attention to hydraulic conditions for a correct working of the system and all those parameters concerning physics and biochemical phenomena: Stochiometric and Kinetics.

Another important aspect treated in following paragraphs is the effect of the external parameters connected to the environment or weather conditions which take part in the system as efficiency modifiers acting directly to biodegrading bacteria.

These efficiency modifiers influence the intrinsic characteristics causing a huge interference. They might change even the main biological phenomena of the system reducing or increasing bacteria activities.

Several years of biology and environmental researching leaded to the definition of all those parameters needed to a well removal processes description. In this regard, the Respirometric Test was designated as the laboratory experiment to be carried out in order to extrapolate experimental data on which to base targeted research on bacterial activity in CWs.
4.1 Hydraulic of CWs

In Constructed Wetlands occurs many processes due to the interaction among substrate, microorganism, bed, plants and all those elements involved in pollutant removal. The “efficiency” is largely influenced by the Hydraulic Retention Time (HRT), which is an indicator of how much time substrate a microorganism remains in contact in order to provide perfect conditions for biochemical reactions, in fact the efficiency is related also on the dynamic of water flow. In fact, if wastewater velocity is too high, some biochemical reactions cannot occur because of an insufficient HRT; many of these removal processes need time to be completed.

With this regard is necessary to involve hydraulics behavior as a new component to design in Constructed Wetlands.

It is therefore necessary to introduce a variable that takes into account the hydraulic characteristics and permeability of the system. For this purpose, HRT was introduced which, as explained above, indicates the time it takes for a particle of fluid to pass through the entire porous media.

The experimental measurement of this parameter takes place by means of the Tracer Test, where an inert tracer is released that flows following the path of the water through the wetland (KADLEC & WALLACE, 2009).

4.2 COD Fractioning

Chemical Oxygen Demand represent the amount of oxygen needed for a total chemical oxidation of organic and inorganic matters in a water sample.
In other words, it is the parameter which give a correlation between substrate, biomass and oxygen consumed.

For a more correct estimation of mathematical models, it is necessary to accurately characterize the wastewater entering the system.

A precise description of the composition of the wastewater ensures a greater ability to predict the models.

It is problematic to apply advanced models when you do not have a sufficiently detailed knowledge.

The COD is divided into fractions that have a precise physical meaning and plant engineering. The breakdown of the COD was initially proposed in the model ASM No. 1 and is now internationally recognized and adopted.

The total COD can be divided into fractions both based on physico-chemical and biological characteristics. As regards the physico-chemical fractionation (as shown in Figure below), the total COD differs in:

- sediment fraction;
- colloidal fraction;
- soluble fraction.

![Fig. 4.1 Fraction of total Chemical Oxygen Demand](image_url)
More specifically the COD is fractionable in many subgroups in base of the biodegradable component:

![Diagram showing wastewater COD fraction]

**Fig. 4.2 Wastewater COD Fraction**

The first large fractionation however divides the biodegradable COD from the non-biodegradable COD and then divides the biodegradable part into fractions which are associated with an oxidation rate and a specific process of biological degradation.

Note: The distinction between Fast and Slowly Biodegradable is based on removal kinetics. Generally, readily biodegradable substrates in batch tests under low $S_0/X_0$ conditions (with negligible biomass growth) are removed in a few hours or fractions while Slowly biodegradable substrates require times ranging from one to more days.
1. **RBCOD (Readily Biodegradable COD):** is the fraction consisting of rapidly biodegradable soluble substrates (acetate, glucose, ethanol, etc.). RBCOD generally constitutes about 10-20% of the total COD of an unsettled civil wastewater. Of this fraction, VFA (in particular acetate) account for 50-70% (Ziglio, Andreottola, Foladori, & Ragazzi, 2001).

2. **SBCOD (Slowly Biodegradable COD):** [slowly biodegradable particulate substrate, from the rapidly hydrolysable portion of the particulate substrate RHCOD (Rapidly Hydrolysable COD) and the colloidal substrate fraction]. It generally constitutes the predominant part of the biodegradable fraction (XS) of COD and consists of complex molecules that must be hydrolyzed before being assimilated by microbial cells. Normally SBCOD fractions between 40 and 60% are obtained. In the ASM models, SBCOD is identified by a single state variable defined as XS, which in general also includes part of the rapidly hydrolysable fraction 1 and the colloidal fraction that is rapidly bio-flocculated by the active sludge and then hydrolyzed. The rapidly hydrolysable fraction, however, is present both in the soluble phase and in the solid phase and generally in the ASM models it can be incorporated in the RBCOD fraction.

3. **NON-BIOODEGRADABLE PARTICULAR COD (XI):** the inert particulate fraction consists of complex sedimentable molecules which are not attacked by hydrolytic enzymes, but which accumulate in activated sludge tanks. It is normally between 10 and 20% of the total COD. The determination of the XI fraction is fundamental for the design as it is decisive in the estimation of sludge production.

4. **NON-BIOODEGRADABLE SOLULE COD (SI):** represents the sum of soluble non-biodegradable organic substances that inevitably end up in the final effluent of a purification plant. Its determination is equivalent to defining the performance of the plant on COD under stable conditions of solid-liquid separation. The percentage in civil sewage is between 2 and 15% of the total COD.
5. **ACTIVE BIOMASS.** The active biomass fraction of COD is given by the sum of the heterotrophic fraction (XH) and the autotrophic fraction (XA), contained in the wastewater and produced by the biological purification processes that take place in the sewer system. While the autotrophic fraction is always negligible, the heterotrophic fraction can be consistent and around 15% of the total COD.

4.3 **TEMPERATURE**

The fundamental physical parameter in the modeling of bacterial growth is temperature. It mainly influences the speed of ration in CWs by decreasing the activity of bacteria and changing the efficiency of the system.

Over the years it has been experimentally verified that the effect of temperature on process speeds can be considered using an Arrhenius law in the definition of a multiplier coefficient for the coefficients of air velocity (kA) and volume (kV).

\[ k_T = k_{20} \cdot \theta^{(T-20)} \]

Dove:

- \( k_T \) = rate coefficient at water temperature T
- \( k_{20} \) = rate coefficient at water temperature 20°C
- \( T \) = temperature of water, °C
- \( \theta \) = modified Arrhenius temperature factor, dimensionless

As the formula above shows, if “modified Arrhenius temperature factor” is 1.0 it indicates that pollutant removal rate is not influenced by the temperature. A \( \theta \)-value greater than 1.0 indicates that k increases with increasing water temperature. A \( \theta \)-value less than 1.0 indicates that k decreases with increasing water temperature.
Rate coefficients that have been temperature-corrected are generally referred to as modified first-order rate coefficients.

For example, from a design point of view a three-time decreasing of removal rate correspond to a three-time increasing of area or volume that CW requires.

If the opposite situation occurs, i.e. the water temperature is above 20°C, the reactions will proceed at a faster rate. It is therefore important to acquire information about the water temperature (not the air temperature) to be used in the design phase.

The average monthly water temperatures of the coldest month are used, so as to be conducive to design safety. In other cases, minimum annual water temperatures are used (Dotro, Günter, Molle, Nivala, & Puigagut, 2017).

4.4 Respirometric Test

Respirometric tests consist in measuring the consumption of oxygen in a reactor in which organic material and a certain amount of biodegradable material are stored. The initiation of biodegradation processes due to bacterial activity will cause a consequent decrease in the concentration of oxygen in the water, this progressive decrease is measured by a sensor that measures the DO (dissolved oxygen).

Closed Respirometer shown in the figure is made up of the following components:

- hermetically sealed reactor equipped with openings for the introduction of the oximetry probe, substrate and oxygen diffusion tube;
- galvanic or polarographic oximetry probe connected to oximeter with data storage system acquired;
- magnetic stirrer rotating for mixing continuous of the sample of active sludge;
- compressor for the oxygenation of the sample through porous micro-diffuser.

Performing respirometric tests requires sampling of biomass directly in the biological oxidation compartment of the plant whose biological kinetics are to be studied. The biomass must be subjected to aeration of at least 6-8 hours, in order to give rise to almost complete degradation of the COD and degradable ammoniacal nitrogen.

The respect of these conditions makes it possible to overlook, during the non-aeration phase, the transfer of oxygen from the small volume of the gaseous phase to the volume of liquid, making the measurement of the coefficient superfluous of KL a transfer and legitimizing the calculation of the breathing speed OUR through oxygen concentration data only dissolved.
5 COMPARISON OF COMMONLY USED MATHEMATICAL MODELS

One of the major difficulties of this research topic is the ability to create a mathematical model that can describe all the biological processes in place and their influence on concentrations of pollutants still present in the system. The kinetics of pollutant removal are very complex and often incomplete, this presents a further difficulty as the system changes depending on the operating conditions. Another fundamental aspect in the mathematical modelling of the kinetics of pollutant removal is the ability to consider all the real biological processes that occur.

It is therefore difficult to think of being able to create such a complex model without the aid of instruments capable of accurately measuring the concentrations of each single biological and non-biological element that takes part in the system, therefore the mathematical models described in this chapter, which are those used in professional practice and research, have a rather simple structure from the mathematical point of view, while from the biological point of view they try to simulate the main chemical-biological reactions directly and indirectly related to the rate of oxygen consumption (Oxygen Uptake Rate - the only experimental data easily obtained from the measurements of dissolved oxygen in respirometric tests conducted in the laboratory).

5.1 ACTIVATED SLUDGE MODELS

Among the first mathematical models developed were the Activated Sludge Models in 1983 proposed by International Association for Water Quality (IAWQ), now called International Water Association (IWA).

They were introduced to simulate bacterial activity within activated sludge in wastewater treatment methods.
In the research of the Constructed Wetlands, however, the same models are used as for the activated sludge because the biochemical reactions of the processes of removal of pollutants are about the same, unless a difference due to the different type of bacterial mass.

Therefore, the aim of the researchers is to modify the ranges of values of the system parameters for the activated sludge and update them to the values resulting from the Constructed Wetlands experimental tests.

**ASM 1**

This model was proposed in order to analyze the processes that occur in a biological purifier using the rations of:

- Carbon oxidation
- Nitrification
- Denitrification

The model's hypotheses are as follows:

- Monod's kinetics to describe the degradation kinetics.
- Death-Regeneration is the type of cycle chosen to describe bacterial life: the metabolism of Death-Regeneration consists in the death of a share of biomass, with production of recyclable materials without oxygen consumption.
- COD is subdivided into two main type of biodegradable substrate: Rapidly biodegradable (soluble Ss), simple molecules able to cross the cell membrane and be immediately available; Slowly biodegradable (Xs particulate matter), complex molecules that require fractionation by extracellular enzymes (hydrolysis) before crossing the cell membrane.
The model is presented below:

![Fig. 5 ASW1 mode scheme](image)

<table>
<thead>
<tr>
<th>Component</th>
<th>Process</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>St</td>
<td>So</td>
<td>Xh</td>
<td>Xa</td>
<td>XhH</td>
<td>XhA</td>
<td>Xa</td>
<td>So</td>
<td>Sno</td>
</tr>
<tr>
<td>Aerobic growth of heterotrophs</td>
<td>1</td>
<td>( \frac{1}{Y_H} )</td>
<td>1</td>
<td>( \frac{1 - Y_H}{Y_H} )</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anoxic growth of heterotrophs</td>
<td>2</td>
<td>( \frac{1}{Y_H} )</td>
<td>1</td>
<td>( \frac{1 - Y_H}{2.86 Y_H} )</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aerobic growth of autotrophs</td>
<td>3</td>
<td>1</td>
<td></td>
<td>( \frac{4.57}{Y_A} + 1 )</td>
<td>( \frac{1}{Y_A} )</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>'Decay' of heterotrophs</td>
<td>4</td>
<td>( 1 - \mu H )</td>
<td>( -1 )</td>
<td>( \mu H )</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>'Decay' of autotrophs</td>
<td>5</td>
<td>( 1 - \mu H )</td>
<td>( -1 )</td>
<td>( \mu H )</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ammonification of soluble organic nitrogen</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>'Hydrolysis' of entrapped organics</td>
<td>7</td>
<td>1</td>
<td>( -1 )</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>'Hydrolysis' of entrapped organic nitrogen</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Observed Conversion Rates [ML⁻¹·T⁻¹]**

\[ \eta = \sum_j \nu_j \rho_j \]

**Stochiometric Parameters**

- Heterotrophic yield: \( Y_H \)
- Autotrophic yield: \( Y_A \)
- Fraction of biomass yielding particulate products: \( \mu H \)
- Mass N/Mass COD in biomass: \( \phi_N \)
- Mass N/Mass COD in products from biomass: \( \phi_A \)

**Kinetic Parameters**

- Heterotrophic growth and decay: \( \mu_H, K_N, K_S, K_{O_H} \)
- Autotrophic growth and decay: \( \mu_A, K_N, K_A, K_{O_A} \)
- Correction factor for anoxic growth of heterotrophs: \( \eta_R \)
- Ammonification: \( k_A \)
- Hydrolysis: \( \eta_H, K_X \)
- Correction factor for anoxic hydrolysis: \( \eta_H \)

**Alkalinity - Molar units**

Description of fundamental processes involved in ASM 1

**Aerobic Growth of Heterotrophic bacteria:** It describes the aerobic bacterial growth of heterotrophic biomass using soluble substrate and dissolved oxygen, so the concentration of both these two elements in the system is discriminatory in limiting the growth rate (for example, a very low concentration of dissolved oxygen severely limits the process up to its cancellation).

**Anoxic Growth of Heterotrophic bacteria:** It analyses the anoxic growth of the heterotrophic biomass using the soluble substrate. The kinetics is similar to the previous one, in fact the coefficient of "half-saturation" is the same, but the maximum speed of the reaction is lower, so the kinetics is pre-multiplied by a factor < 1.

**Aerobic Growth of Autotrophic bacteria:** Describes the aerobic growth of autotrophic biomass. This reaction transforms ammoniacal nitrogen into nitrate, which serves as a feedstock for autotrophic bacteria.

**Heterotrophic Decay:** it describes the Heterotrophic biomass decay according to the initial hypothesis of Death – Regeneration cycle. This process is not influenced by the external environmental conditions, thus the cycle continue as long as the biomass remain in the reactor.

**Autotrophic Decay:** The same process as the previous one but for autotrophic biomass.
Ammonification of soluble organic nitrogen: Describes the transformation of biodegradable organic nitrogen into ammoniacal nitrogen through first-order empirical expression.

Description of parameters involved in ASM 1

Fig. 5.2 ASM 1 Definition of Parameters
Disadvantages of ASM 1:

1. Soluble organic nitrogen is difficult to measure.
2. Nitrification kinetics cannot be realistically quantified.
3. Organic particulate matter in ASM 1 is subdivided according to its origin, this is impossible.
4. The evaluation of the parameters of hydrolysis processes, reactions with an important consumption of oxygen and nitrogen, is very complex.
5. Difficulties in the evaluation of kinetic parameters due to the introduction of lysis processes.
6. Does not consider the possibility of introducing different rates of decay of nitrifiers in conditions of oxygen deficiency or richness. This creates problems over and above in determining the maximum nitrification rate with high Sludge Retention Time.

ASM 2

The "Activated Sludge Model No. 2" is simply an extension of the ASM 1. Phosphorus removal processes (2 chemical processes for phosphorus precipitation) are also introduced here.

Common assumptions with ASM 1:

- constant kinetic parameters
- hydrolysis processes are coupled and simultaneous.

Fundamental processes:

*Aerobic hydrolysis*: aerobic hydrolysis of the substrate slowly biodegradable which characterize hydrolysis under aerobic conditions.
**Anoxic hydrolysis**: anoxic hydrolysis of the substrate slowly biodegradable which characterizes hydrolysis under anoxic conditions. Slower than aerobic hydrolysis.

**Anaerobic hydrolysis**: anaerobic hydrolysis of the substrate slowly. Biodegradable characterizing anaerobic hydrolysis

**Aerobic growth on SF**: represents the aerobic growth of the microorganisms on fermentable substrate SF.

**Aerobic growth on SA**: it represents the aerobic growth of the microorganisms on a SA fermentable substrate, we assume the same speed $\mu m$ and yield coefficients $YH$. Both processes require oxygenSO2. SNH4 and SPO4 nutrients, SALK alkalinity and produce solids suspended XTSS.

**Anoxic growth on SF - Denitrification**: represents the anoxic growth of heterotrophic microorganisms on substrate fermentable, requires SNO3 nitrate as an electron acceptor. Assumes that all SNO3 nitrate is reduced to SN2 nitrogen. The denitrification releases alkalinity, it is assumed to be inhibited from oxygen and the maximum rate of growth $\mu m$ is lower than the aerobic case by the coefficient $hNO3$.

**Anoxic growth on SA - Denitrification**: similarly to the previous process represents the anoxic growth of the heterotrophic micro-organisms on fermentation products SA.

**Fermentation**: indicates fermentation, under anaerobic conditions is assumes that heterotrophic organisms are capable of carrying out the fermentation in which SF, an easily degradable substrate, is transformed into SA fermentation products. It is introduced as simple process of transformation.

**Lysis**: represents the lysis of heterotrophic organisms and is modeled similarly to the ASM1.

**XPHA accumulation**: Task Group suggested a model simplified to characterize the PAOs, making some severe restrictions. The model assumes that these bodies cannot denitrify and can only grow on organic material stored XPHA inside the cell.
**XPP accumulation**: represents the accumulation of polyphosphates: the accumulation of SPO4 orthophosphates in the form of internal cell polyphosphate, XPP, requires the PAOs to derive energy, which can be obtained from the breathing of XPHA. Regeneration of polyphosphates is a requirement for the growth of PAOs, because the organic substrate SA is accumulated only after the release of the polyphosphates. If the phosphorus content is the accumulation is blocked, therefore a term is introduced inhibitor that becomes active when the value of the XPP/XPAO ratio reaches a maximum permissible value Kmax.

**Aerobic growth of XPAO**: describes the growth of PAOs, assumes that grow only at the expense of XPHA Phosphorus is continuously issued by the lysis of XPP, therefore it can be assumed that the bodies consume SPO4 orthophosphate as a nutrient for biomass production. The growth of PAOs is shaped as a process obligatorily aerobic.

**XPAO lysis**: represents the lysis of PAOs.

**XPP lysis**: represents the lysis of accumulation products.

**XPHA Lysis**: Represents the lysis of accumulation products.

**XA Aerobic Growth**: Represents the growth of organism nitrifiers. These are obligatorily aerobic organisms, consume ammonia as a substrate and nutrient and produce nitrate, decreasing the alkalinity. The process is modelled in such a way similar to ASM1.

**XA Lysis**: represents the lysis of nitrifying organisms and is modelled in a similar way to ASM1.

**Phosphate precipitation**

**Phosphate Reduction**
Limitation of the model:

The model has restrictions in its application especially from the point of view of phosphorus storage organisms (PAOs) which cannot be perfectly analyzed.

In addition, ASM 2 has difficulties in simulating removal processes if the water temperature is not within a set range (10° - 25° (Simarosti, 2004)), the pH must be close to the neutral value (7). This model is suitable for domestic wastewater but does not consider the separation of solids in sedimentation tanks.

ASM 3

The ASM 3 is a model created as an update of the ASM 1 to obviate some aspects dealt with in the first model that set substantial limits in the calibration of the parameters describing some removal processes.

Moreover, the turning point that this model provides is the decoupling of some variables facilitating the calibration of the kinetic and stoichiometric parameters of the system.

The main processes remain equal to those involved in ASM 1:

- Oxygen consumption
- Sludge production
- Nitrification and denitrification

Soluble components are marked with letter S; Particulate components are marked with letter X.

Therefore, ASM 3 only considers biological transformation processes without involving chemical precipitation processes.
The following tables show the main chemical-biological components involved in the system and the fundamental processes that occur in the reactors (every following figures are taken from (Gujer, Henze, Mino, & Loosdrecht, 1999)):

<table>
<thead>
<tr>
<th>Dissolved components</th>
</tr>
</thead>
<tbody>
<tr>
<td>$S_0$</td>
</tr>
<tr>
<td>$S_1$</td>
</tr>
<tr>
<td>$S_5$</td>
</tr>
<tr>
<td>$S_{NH}$</td>
</tr>
<tr>
<td>$S_{N2}$</td>
</tr>
<tr>
<td>$S_{NO}$</td>
</tr>
<tr>
<td>$S_{HCO}$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Particulate components</th>
</tr>
</thead>
<tbody>
<tr>
<td>$X_1$</td>
</tr>
<tr>
<td>$X_5$</td>
</tr>
<tr>
<td>$X_H$</td>
</tr>
<tr>
<td>$X_{STO}$</td>
</tr>
<tr>
<td>$X_A$</td>
</tr>
<tr>
<td>$X_{TS}$</td>
</tr>
</tbody>
</table>

**Fig. 5.3 Definition of all components involved in domestic wastewater treatment.**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Characterization</th>
</tr>
</thead>
<tbody>
<tr>
<td>$f_{S1}$</td>
<td>Production of $S_1$ in hydrolysis</td>
</tr>
<tr>
<td>$Y_{STO,02}$</td>
<td>Aerobic yield of stored product per $S_5$</td>
</tr>
<tr>
<td>$Y_{STO,NO}$</td>
<td>Anoxic yield of stored product per $S_5$</td>
</tr>
<tr>
<td>$Y_{H02}$</td>
<td>Aerobic yield of heterotrophic biomass</td>
</tr>
<tr>
<td>$Y_{HNO}$</td>
<td>Anoxic yield of heterotrophic biomass</td>
</tr>
<tr>
<td>$Y_A$</td>
<td>Yield of autotrophic biomass per NO$_3$-N</td>
</tr>
<tr>
<td>$i_{NS1}$</td>
<td>N content of $S_1$</td>
</tr>
<tr>
<td>$i_{NSS}$</td>
<td>N content of $S_5$</td>
</tr>
<tr>
<td>$i_{NX1}$</td>
<td>N content of $X_1$</td>
</tr>
<tr>
<td>$i_{NXS}$</td>
<td>N content of $X_5$</td>
</tr>
<tr>
<td>$i_{NBM}$</td>
<td>N content of biomass, $X_H$, $X_A$</td>
</tr>
<tr>
<td>$i_{TSX1}$</td>
<td>TSS to COD ratio for $X_1$</td>
</tr>
<tr>
<td>$i_{TSXS}$</td>
<td>TSS to COD ratio for $X_5$</td>
</tr>
<tr>
<td>$i_{TSBM}$</td>
<td>TSS to COD ratio for biomass, $X_H$, $X_A$</td>
</tr>
<tr>
<td>$i_{TSRO}$</td>
<td>TSS to COD ratio for $X_{STO}$ based on PHB</td>
</tr>
</tbody>
</table>

**Fig. 5.4 Stochiometric and composition parameter for ASM 3.**
The following figures show the Kinetics Parameters for Activated Sludge Model 3:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_H$</td>
<td>Hydrolysis rate constant</td>
</tr>
<tr>
<td>$K_X$</td>
<td>Hydrolysis saturation constant</td>
</tr>
</tbody>
</table>

**Fig. 5.5 Kinetic Parameters for hydrolysis processes**

**Heterotrophic organisms, denitrification, $X_H$**

- $k_{STO}$: Storage rate constant
- $\eta_{NO}$: Anoxic reduction factor
- $K_O$: Saturation constant for $S_O$
- $K_{NO}$: Saturation constant for $S_{NO}$
- $K_E$: Saturation constant for substrate $S_E$
- $K_{STO}$: Saturation constant for $X_{STO}$
- $\mu_H$: Heterotrophic max. growth rate
- $K_{NH}$: Saturation constant for ammonium, $S_{NH}$
- $K_{HCO}$: Bicarbonate saturation constant of $X_H$
- $b_{H,O2}$: Aerobic endogenous respiration rate of $X_H$
- $b_{H,NO}$: Anoxic endogenous respiration rate of $X_H$
- $b_{STO,O2}$: Aerobic respiration rate for $X_{STO}$
- $b_{STO,NO}$: Anoxic respiration rate for $X_{STO}$

**Fig. 5.6 Kinetics Parameters for Heterotrophic organism, Denitrification, $X_H$**

**Autotrophic organisms, nitrification, $X_A$**

- $\mu_A$: Autotrophic max. growth rate of $X_A$
- $K_{A-NH}$: Ammonium substrate saturation for $X_A$
- $K_{A-O}$: Oxygen saturation for nitrifiers
- $K_{A-HCO}$: Bicarbonate saturation for nitrifiers
- $b_{A,O2}$: Aerobic endogenous respiration rate of $X_A$
- $b_{A,NO}$: Anoxic endogenous respiration rate of $X_A$

**Fig. 5.7 Kinetics Parameters for Autotrophic organism, Nitrification, $X_A$**
<table>
<thead>
<tr>
<th>j</th>
<th>Process</th>
<th>Process rate equation $p_i$, all $p_i &gt; 0$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hydrolysis</td>
<td>$k_H \frac{X_S / X_H}{K_X + X_S / X_H} X_H$</td>
</tr>
<tr>
<td>2</td>
<td>Aerobic storage of COD</td>
<td>$k_{STD} \frac{S_O}{K_O + S_O} \frac{S_S}{K_s + S_s} X_H$</td>
</tr>
<tr>
<td>3</td>
<td>Anoxic storage of COD</td>
<td>$k_{STD} \frac{n_{NO}}{K_O + S_O} \frac{S_NO}{K_NO + S_NO} \frac{S_S}{K_s + S_s} X_H$</td>
</tr>
<tr>
<td>4</td>
<td>Aerobic growth</td>
<td>$k_H \frac{S_O}{K_O + S_O} \frac{S_HH}{K_H + S_HH} \frac{S_HCO}{K_HCO + S_HCO} \frac{X_{STO} / X_H}{X_{STO} / X_H} X_H$</td>
</tr>
<tr>
<td>5</td>
<td>Anoxic growth (denitrification)</td>
<td>$k_H \frac{n_{NO}}{K_O + S_O} \frac{S_NO}{K_NO + S_NO} \frac{S_HH}{K_HH + S_HH} \frac{S_HCO}{K_HCO + S_HCO} \frac{X_{STO} / X_H}{X_{STO} / X_H} X_H$</td>
</tr>
<tr>
<td>6</td>
<td>Aerobic endogenous respiration</td>
<td>$b_{H,02} \frac{S_O}{K_O + S_O} X_H$</td>
</tr>
<tr>
<td>7</td>
<td>Anoxic endogenous respiration</td>
<td>$b_{H,NO} \frac{K_O}{K_O + S_O} \frac{S_NO}{K_NO + S_NO} X_H$</td>
</tr>
<tr>
<td>8</td>
<td>Aerobic respiration of $X_{STO}$</td>
<td>$b_{STO,02} \frac{S_O}{K_O + S_O} X_{STO}$</td>
</tr>
<tr>
<td>9</td>
<td>Anoxic respiration of $X_{STO}$</td>
<td>$b_{STO,NO} \frac{K_O}{K_O + S_O} \frac{S_NO}{K_NO + S_NO} X_{STO}$</td>
</tr>
<tr>
<td>10</td>
<td>Nitrification</td>
<td>$k_A \frac{S_O}{K_A,O + S_O} \frac{S_HH}{K_A,H + S_HH} \frac{S_HCO}{K_A,HCO + S_HCO} X_A$</td>
</tr>
<tr>
<td>11</td>
<td>Aerobic endogenous respiration</td>
<td>$b_{A,02} \frac{S_O}{K_O + S_O} X_A$</td>
</tr>
<tr>
<td>12</td>
<td>Anoxic endogenous respiration</td>
<td>$b_{A,NO} \frac{K_O}{K_O + S_O} \frac{S_NO}{K_NO + S_NO} X_A$</td>
</tr>
</tbody>
</table>

Fig. 5.8 Kinetics equations for each ASM 3 processes
Comparison between ASM 1 and ASM 3

To understand the importance of the introduction of the ASM 3 model, it is necessary to analyze the changes that have been made compared to previous models.

The process of accumulation in the heterotrophic metabolism of the organic matter, the main difference compared to ASM 1, is highlighted. The introduction of this process changes the way of evaluating some biological phenomena previously evaluated by kinetic equations. The processes of hydrolysis and are removed from the system of equations and incorporated into the kinetics of decay and growth.
Bacterial growth therefore no longer occurs directly from the consumption of rapidly biodegradable substrate (Sₜ) but is first stored as a cellular component (Xₜ) and subsequently used consumed for bacterial growth.

This model is therefore very easy to calibrate the parameters as the various processes are decoupled and the computational costs can be easily carried out by any computer. This conclusion has also led to the introduction of Endogenous Breathing in which all the processes of decay and lysis are put, this because endogenous breathing is more easily correlated to the experimental data observed.

![Flow of COD in ASM1 and ASM3](Gujer W., Henze, Mino, & Loosdrecht, 2000).

Figure 5.10 shows the flow of the COD in ASM 1 and ASM 3; as it sees, Nitrifiers and Heterotrophs are independent in terms of COD. The readily substrate is first stored and then used for bacteria growth, this means that as long as substrate is still in the system, OUR and oxygen consumption are due only to respiration and storage process until the depletion of Sₜ.
5.2 SIMULTANEOUS STORAGE AND GROWTH

Subsequently, over the years and research in this field, it was noted that the ASM 3 model had shortcomings. Some researchers believed that the various changes made to the original model were incomplete because at the biological level there were still processes not considered.

The evolution of the ASM 3 model in Simultaneous Storage And Growth was proposed by Sin in the publication (Sin, et al., 2005) in which is written:

“By critically evaluating previous models, a new mechanistic model is developed to describe simultaneous storage and growth processes occurring in activated sludge systems under aerobic conditions. Identifiability was considered an important criterion during the model development since it among others helps to increase the reliability and applicability of models to full-scale WWTPs.”

Model proposed by (Fan, A. Vanrolleghem, Lu, & Qiu, 2012).

In this paragraph is presented the modified version of ASM 3, proposed by (Fan, A. Vanrolleghem, Lu, & Qiu, 2012), which involves Simultaneous Storage and Growth processes.

In order to better explain the pathways of COD among each biological reactions in the Constructed Wetlands, the mentioned researchers have introduced in the mathematical model, developed by themselves, the removal processes concerning “Growth on Storage products” also during the Feast Phase, while substrate is still present in the reactor.
In their opinion, the following model should provide a better way to describe biological phenomena occurring in Constructed Wetlands.

As already explained, compared to ASM 3 this model differs from the process of direct growth on the substrate under aerobic conditions, is also excluded the nitrification reaction because the nitrifiers are inhibited by the presence of AHU.

The images below are taken from the original publication (Fan, A.Vanrolleghem, Lu, & Qiu, 2012) and show the model in matrix form and the parameters with their respective values used:

![Fig. 5.11 Matrix Model Equations of Simultaneous Storage and Growth](image)

**Definitions of Matrix Components:**

“$M_D$” referee to Monod’s kinetic of oxygen removal rate:

$$\frac{S_{O_2}}{S_{O_2} + K_{O_2}}$$

Kinetic expression of Substrate Storage Rate $[\text{Eq. (1)}]$:

$$f_{STO} \times q_{MAX} \times Y_{STO} \times \frac{S_{O_2}}{S_{O_2} + K_{O_2}} \times \frac{S_{S}}{S_{S} + K_{S}} \times X_{H}$$

Kinetic expression of Direct Aerobic Growth on Substrate Rate $[\text{Eq. (2)}]$:

$$(1 - f_{STO}) \times q_{MAX} \times Y_{H,S} \times \frac{S_{O_2}}{S_{O_2} + K_{O_2}} \times \frac{S_{S}}{S_{S} + K_{S}} \times X_{H}$$
Kinetic expression of Growth on Storage Products [Eq. (3)]:

$$\mu_{MAX,STO} \times \frac{S_{O_2}}{S_{O_2} + K_{O_2}} \times \frac{X_{STO}/X_H}{K_{STO}/X_H} \times X_H$$

The following table represents

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Y_{STO}$</td>
<td>Yield coefficient for storage, gCOD$<em>{X</em>{STO}}$/gCOD$_{S_S}$</td>
<td>0.8</td>
</tr>
<tr>
<td>$Y_{HS}$</td>
<td>Yield coefficient for growth on $S_S$, gCOD$<em>{X</em>{H}}$/gCOD$_{S_S}$</td>
<td>0.6</td>
</tr>
<tr>
<td>$Y_{H,STO}$</td>
<td>Yield coefficient for growth on $X_{STO}$, gCOD$<em>{X</em>{H}}$/gCOD$<em>{X</em>{STO}}$</td>
<td>0.68</td>
</tr>
<tr>
<td>$q_{MAX}$</td>
<td>Maximum substrate uptake rate, per day</td>
<td>1.2</td>
</tr>
<tr>
<td>$f_i$</td>
<td>Fraction of $X_i$ in decay, gCOD$<em>{X_i}$/gCOD$</em>{X_{IM}}$</td>
<td>0.2</td>
</tr>
<tr>
<td>$f_{STO}$</td>
<td>Fraction of substrate used for storage, gCOD$<em>{X</em>{STO}}$/gCOD$_{S_S}$</td>
<td>0.6</td>
</tr>
<tr>
<td>$K_{STO}$</td>
<td>Storage products affinity constant, gCOD$<em>{X</em>{STO}}$/m$^3$</td>
<td>0.024</td>
</tr>
<tr>
<td>$\mu_{MAX,STO}$</td>
<td>Maximum growth rate on $X_{STO}$, per day</td>
<td>2</td>
</tr>
<tr>
<td>$K_S$</td>
<td>Substrate affinity constant, gCOD$_{S_S}$/m$^3$</td>
<td>0.7</td>
</tr>
<tr>
<td>$K_O$</td>
<td>DO affinity constant, gO$_2$/m$^3$</td>
<td>0.2</td>
</tr>
<tr>
<td>$b_H$</td>
<td>Biomass endogenous decay rate coefficient, per day</td>
<td>0.2</td>
</tr>
<tr>
<td>$b_{STO}$</td>
<td>Endogenous decay rate coefficient of $X_{STO}$, per day</td>
<td>0.2</td>
</tr>
</tbody>
</table>

*Fig. 5.12 Model’s Kinetic and Stochiometric Parameters*
6 Respirometric Tests Performed in Laboratory

The aim of this work is to calibrate the parameters of the model adopted to describe the experimental graphs conducted in the Environment Laboratory of the Department of Civil and Environmental Engineering, IST University of Lisbon.

The activities foreseen for this thesis project are divided into two phases: the first phase of acquisition of respirometric data in the laboratory, the second phase of study of the model used and calibration of the parameters for a good description of the empirical graphs of OUR.

The way in which the experiments were performed is divided into two methodologies. The main difference is the acquisition of data from experiments conditioned and not conditioned by the hydraulics of the system:

- In the first methodology, the organic material is extracted from the bed of the wetland. Subsequently introduced in a container with water and agitated, this to remove the organic material from the gravelly sandy material and thus obtain a water solution of biofilm. The experimental tests are therefore conducted on this solution and therefore show the behavior of the system (behavior of the bacteria) in the absence of the hydraulic conditions present in a real situation.

- In the second methodology, instead, the hydraulic conditions, represented by the porous medium in which the organic material has developed, influence the system directly. The material on which the tests will be carried out is also taken directly from the wetlands, purified of worms, algae, wooden sticks and any element that may in some way influence the porosity, and therefore the hydraulics, of the system.
6.1 DESCRIPTION OF THE BEDS AND SYNTHETIC SEWAGE COMPOSITION

In the hydraulics laboratory of the Instituto Superior Técnico (IST), beds for the experimental simulation of an HSSF Constructed Wetland have been installed. These beds serve as incubators for bacterial growth and proliferation of biofilms, which will then be used for respirometric tests.

The beds dimensions are 1.1 m x 0.71 m x 0.76 m (length x width x height). They are filled with 35 cm of gravel (4-8 mm, 30% porosity) and water, up to 5 cm below the gravel surface.

Phragmites Australis vegetation was planted in 2010 and fed with synthetic sewage. Composition of Synthetic Sewage is reported in following table (Galvão & Matos, 2012):

<table>
<thead>
<tr>
<th>Synthetic Sewage component</th>
<th>Quantity (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea</td>
<td>6</td>
</tr>
<tr>
<td>Na-Acetate</td>
<td>13.2</td>
</tr>
<tr>
<td>Peptone</td>
<td>1.7</td>
</tr>
<tr>
<td>Starch</td>
<td>12.2</td>
</tr>
<tr>
<td>Powdered milk</td>
<td>11.2</td>
</tr>
<tr>
<td>Soy oil</td>
<td>2.9</td>
</tr>
<tr>
<td>Fertilizer solution</td>
<td>300 (mL)</td>
</tr>
</tbody>
</table>

*Tab. 6.1 Synthetic sewage compounds with related concentrations*

With synthetic sewage presented above, beds were been feed for 3 years, from 2010 to 2017.
6.2 FIRST RESPIROMETRIC TEST METHODOLOGY

The first type of respirometer used for experimental tests is called LSS and consists of measurements in the liquid phase, static gas and static regime of reactor operation (Spanjers, 1996).

At the Instituto Superior Técnico (IST), University of Lisbon, Portugal, a respirometer of this type composed of:

- Main reactor: where continuous ventilation takes place to prevent oxygen from becoming a limiting factor;
- Compressor: to provide the right aeration;
- Hydraulic pump: allows the circulation of water inside the dissolved oxygen measurement system;
- Turbine stirrer: completely mixes the solution;
- Dissolved Oxygen probe (DO).

The cycle time of a test includes an inactivity period of 4 minutes without recirculation, static liquid within the respirometric cell, and a pumping period of 2 minutes, ensuring sample renewal and DO within the cell (Pisoeiro, Galvão, Pinheiro, Ferreira, & Matos, 2017).

Fig. 6.1 Schematic representation of first type of Respirometry
6.3 Second Respirometric Test Methodology

The second type of respirometer used for experimental tests was developed in Instituto Superior Técnico (IST), University of Lisbon, Portugal, too.

The composition of this respirometer is presented below:

- **Main reactor**: in which gravelly material, including biofilm, is stored directly from the beds;
- **Hydraulic pump**: to allow the liquid to circulate and be subjected to the necessary measurements;
- **Compressor**: to maintain the concentration of dissolved oxygen at the inlet close to saturation;
- **Due Dissolved Oxygen probe (DO)**

*Fig. 6.2 Photo of the Respirometry described in this paragraph.*
6.4 Bed Feeding

The mass loads COD (ml), during the various feeding periods of the beds, are varied from 7.0 g COD/(m².day) and 35.2 g COD/(m².day) to analyze the behavior of the biomass for different mass loading speeds [Galvão et al. (article work in progress) - Storage mechanisms in biofilms from constructed wetlands].

The main phases are reported as follows:

<table>
<thead>
<tr>
<th>Phase</th>
<th>COD Mass Load (g/(m².day))</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>7.0 (1.0)</td>
</tr>
<tr>
<td>B</td>
<td>15.9 (2.5)</td>
</tr>
<tr>
<td>C</td>
<td>35.2 (5.2)</td>
</tr>
<tr>
<td>D</td>
<td>15.6 (2.2)</td>
</tr>
<tr>
<td></td>
<td>MLin</td>
</tr>
<tr>
<td>A</td>
<td>0.2</td>
</tr>
<tr>
<td>B</td>
<td>0.3</td>
</tr>
<tr>
<td>C</td>
<td>0.8</td>
</tr>
<tr>
<td>D</td>
<td>0.6</td>
</tr>
</tbody>
</table>

Tab. 6.2 COD mass load about feeding beds in various phases.

Each of which is characterized by a range of values of COD mass load reported in the graphic below:

Fig. 6.3 COD mass loading rate (ML) applied to the experimental Wetland during the experimental phases of the study.
6.5 Respirometric test Results

During the research of Ana Galvão e Joana Pioeiro, at IST University of Lisbon, more than 38 experiments, with first respirometric test methodology described in paragraph 6.2, were conducted divided into the various phases of feeding the beds.

The initial concentration of acetate in the main reactor is regulated at each stage to prevent DO levels within the respirometric cell from falling below 3 mg/L, thus ensuring that DO is not a limiting factor.

Phase B is not considered in this work.

Fig. 6.4 Most significant charts from “Phase A” experiments corresponding to the tests of: 08 January 2014 (blue line) and 27 January 2014 (brown line).
Fig. 6.5 Most significant charts from “Phase C” experiments corresponding to the tests of: 07 March 2016 (green line) and 14 March 2016 (yellow line).
Fig. 6.5 Most significant charts from “Phase D” experiments corresponding to the tests of: 10 May 2017 (brown line), 15 May 2018 (grey line) and 02 June 2017 (yellow line).
7 Model Calibration

The work carried out in this thesis project consists in the identification of a mathematical model suitable for the description and analysis of biological phenomena and removal processes that occur within the Wetland.

By means of respirometric tests, the graphs representing the trend of the OUR over time have been identified (see previous chapter). These graphs were used to calibrate the parameters of the mathematical model.

The model used for the description of biological phenomena, which occur in the HSSF Constructed Wetland developed in the laboratory, is of the Simultaneous Storage and Growth type.

In particular, we refer to the model proposed by (Fan, A.Vanrolleghem, Lu, & Qiu, 2012), which seems to be the model closest to the real situation that occurs in a Constructed Wetland.

Below are reported the parameters of the model that were then calibrated during the final phase of the thesis project:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Y_{STO}$</td>
<td>Yield coefficient for storage, gCOD$<em>{X</em>{STO}}$/gCOD$<em>{S</em>{S}}$</td>
</tr>
<tr>
<td>$Y_{HS}$</td>
<td>Yield coefficient for growth on $S_{S}$, gCOD$<em>{X</em>{H}}$/gCOD$<em>{S</em>{S}}$</td>
</tr>
<tr>
<td>$Y_{HSTO}$</td>
<td>Yield coefficient for growth on $X_{STO}$, gCOD$<em>{X</em>{H}}$/gCOD$<em>{X</em>{STO}}$</td>
</tr>
<tr>
<td>$q_{MAX}$</td>
<td>Maximum substrate uptake rate, per day</td>
</tr>
<tr>
<td>$f_i$</td>
<td>Fraction of $X_{i}$ in decay, gCOD$<em>{X</em>{i}}$/gCOD$<em>{X</em>{BM}}$</td>
</tr>
<tr>
<td>$f_{STO}$</td>
<td>Fraction of substrate used for storage, gCOD$<em>{X</em>{STO}}$/gCOD$<em>{S</em>{S}}$</td>
</tr>
<tr>
<td>$K_{STO}$</td>
<td>Storage products affinity constant, gCOD$<em>{X</em>{STO}}$/m$^3$</td>
</tr>
<tr>
<td>$\mu_{MAX,STO}$</td>
<td>Maximum growth rate on $X_{STO}$, per day</td>
</tr>
<tr>
<td>$K_{S}$</td>
<td>Substrate affinity constant, gCOD$<em>{S</em>{S}}$/m$^3$</td>
</tr>
<tr>
<td>$K_{O}$</td>
<td>DO affinity constant, gO$_2}$/ m$^3$</td>
</tr>
<tr>
<td>$b_{H}$</td>
<td>Biomass endogenous decay rate coefficient, per day</td>
</tr>
<tr>
<td>$b_{STO}$</td>
<td>Endogenous decay rate coefficient of $X_{STO}$, per day</td>
</tr>
</tbody>
</table>

Tab. 7.1 Model’s Parameters of (Fan, A.Vanrolleghem, Lu, & Qiu, 2012)
7.1 Calibration method: Least Squares

The method chosen for the calibration of the parameters of the mathematical model is the classic Minimal Square Method.

This approach was chosen because it is very reliable and easily adaptable to the calibration of a model from experimental data.

Since experimental observations are often affected by errors, accidental and systematic, the resulting graph also has errors, called "noises" that make the function "not smooth". Therefore, this suggests that it no longer makes sense to calibrate the model by imposing the equality of the latter to the measured data, as is usually done in the traditional method, but it is acceptable for the model to approach.

As an objective function, therefore, we choose what is defined as the Sum of the Average Quadratic Scraps, that is the sum of the differences between the simulated value and the measured value elevated to the square:

\[ F = \sum_{i=1}^{m} (OUR_{measured,i} - OUR_{simulated,i})^2 \]

Where:

- \( OUR_{measured,i} \) is the \( i \)th value of OUR measured at time \( t_i \)
- \( OUR_{simulated,i} \) is the \( i \)th value of OUR simulated at time \( t_i \)

Calibration, therefore, consists in finding those values of the parameters such that the objective function is minimal (i.e. that the simulated graph is similar to the one measured).

With this purpose, the model has been implemented in a Excel spreadsheet.
The formula below shows the expression of the Oxygen Uptake Rate that binds the parameters and variables of the simulated model:

\[
\text{OUR} = \]

\[
(-1 + Y_{STO}) \times \left( f_{STO} \times q_{MAX} \times Y_{STO} \times \frac{S_{O_2}}{K_{O_2} + S_{O_2}} \times \frac{S_S}{K_S + S_S} \times X_H \right) +
\]

\[
+ \left( 1 - \frac{1}{Y_{HS}} \right) \times \left( (1 - f_{STO}) \times q_{MAX} \times Y_{HS} \times \frac{S_{O_2}}{K_{O_2} + S_{O_2}} \times \frac{S_S}{K_S + S_S} \times X_H \right) +
\]

\[
+ \left( 1 - \frac{1}{Y_{HS,STO}} \right) \times \left( \mu_{MAX,STO} \times \frac{S_{O_2}}{K_{O_2} + S_{O_2}} \times \frac{X_{STO}}{X_{HSTO}} \times \frac{X_H}{X_H} \right) +
\]

\[
+ (-1 + f_i) \times \left( b_H \times \frac{S_{O_2}}{K_{O_2} + S_{O_2}} \times X_H \right) +
\]

\[
+ (-1) \times \left( b_{STO} \times \frac{S_{O_2}}{K_{O_2} + S_{O_2}} \times X_{STO} \right)
\]

The first terms of the previous equations represent the stoichiometric linkage of dissolved oxygen involved in the various biological processes of removal.

The second terms, on the other hand, represent the kinetics of the various biological processes that contribute to the removal of dissolved oxygen.

As a result, therefore, the reverse of the rate of oxygen consumption by heterotrophic bacteria (OUR) is obtained.

Following the suggestions of (Fan, A.Vanrolleghem, Lu, & Qiu, 2012), the model parameters should have the values shown in the following figure (as described in paragraph 5.2):
As shown in the graphic below how the model works on DO data measured:

![Model response graph](image)

*Fig. 7.1 Model response*
After having chosen a time interval of 5 minutes, the observed DO values have been taken directly from the experimental data looking for the above DO value corresponding to the time interval in which the measurement is to be known.

The standard time interval considered is shown, by way of example, in the column below:

<table>
<thead>
<tr>
<th>Time (h)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.083333</td>
</tr>
<tr>
<td>0,083333</td>
<td>0.166667</td>
</tr>
<tr>
<td>0.166667</td>
<td>0.25</td>
</tr>
<tr>
<td>0.25</td>
<td>0.333333</td>
</tr>
<tr>
<td>0.333333</td>
<td>0.416667</td>
</tr>
<tr>
<td>0.416667</td>
<td>0.5</td>
</tr>
<tr>
<td>0.5</td>
<td>0.583333</td>
</tr>
<tr>
<td>0.583333</td>
<td>0.666667</td>
</tr>
<tr>
<td>0.666667</td>
<td>0.75</td>
</tr>
<tr>
<td>0.75</td>
<td>0.833333</td>
</tr>
<tr>
<td>0.833333</td>
<td>0.916667</td>
</tr>
<tr>
<td>0.916667</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>1.083333</td>
</tr>
<tr>
<td>1.083333</td>
<td>1.166667</td>
</tr>
<tr>
<td>1.166667</td>
<td>1.25</td>
</tr>
<tr>
<td>1.25</td>
<td>1.333333</td>
</tr>
<tr>
<td>1.333333</td>
<td>1.416667</td>
</tr>
<tr>
<td>1.416667</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Tab. 7.3 Time interval of 5 minutes chosen for simulations

For the sake of consistency of the units of measurement, during the simulation the time was transformed into "days" as the parameters of the model are also calibrated in days.
7.2 Calibration of Phase D experimental charts

The graphs obtained from the experimental tests during this phase show a particularly interesting and unusual trend, for this reason they were the first on which the calibration was performed.

Initially, the Excel spreadsheet was set up, as indicated in paragraph 7.1.

Subsequently, the model was calibrated using the Excel "Solver" tool. This tool has been instructed to minimize the parameter "F" (Objective Function - specified in paragraph 7.1). This parameter preserves information on the discrepancy between the experimental observations and the simulated model. The fixed parameters that the Solver must vary have been specified, taking care to impose upper and lower limits that the latter can assume, in order to prevent the calibration method from finding values of the parameters that, even if the experimental data are well rented, do not make any physical sense.

GRG non-linear has been selected as the optimization method because all the methods proposed by the Solver converge on the same result.

The main problem, which did not bring the method to an adequate convergence, was found in the definition of the initial values to be given to the parameters. In fact, this method has a strong dependence on the initial values that are assigned to the terms to be optimized.

To overcome this problem, before the assignment of the Initial Values to be given to the parameters to be optimized, Sensitivity Analyses of these parameters were made, approaching as close as possible to the value that brings the simulated graph as close as possible to the one observed. This procedure has been done for each stoichiometric and kinetic parameter of the model, after which, launching the Solver tool, excellent results have been obtained.
Fig. 7.2 Observed OUR (brown line) and Simulated OUR with Calibrated Parameters (blue line) – Input Acetate 149 mgCOD/l

<table>
<thead>
<tr>
<th>Nome</th>
<th>Valore originale</th>
<th>Valore finale</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Y_{H,S}$</td>
<td>0,9</td>
<td>0,986619569</td>
</tr>
<tr>
<td>$Y_{H,STO}$</td>
<td>0,1</td>
<td>0,201545597</td>
</tr>
<tr>
<td>$Y_{STO,S}$</td>
<td>0,8</td>
<td>0,771684767</td>
</tr>
<tr>
<td>$f_{STO}$</td>
<td>0,1</td>
<td>0,06609988</td>
</tr>
<tr>
<td>$f_{i}$</td>
<td>0,6</td>
<td>0,2</td>
</tr>
<tr>
<td>$q_{MAX}$</td>
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<td>7,504600896</td>
</tr>
<tr>
<td>$j_{MAX,STO}$</td>
<td>2,5</td>
<td>4,314476301</td>
</tr>
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<td>$K_{STO}$</td>
<td>3,8</td>
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</tr>
<tr>
<td>$K_{S}$</td>
<td>0,5</td>
<td>7,631581488</td>
</tr>
<tr>
<td>$K_{O}$</td>
<td>0,7</td>
<td>0,763516672</td>
</tr>
<tr>
<td>$b_{H}$</td>
<td>0,01</td>
<td>0,018272089</td>
</tr>
<tr>
<td>$b_{STO}$</td>
<td>0,01</td>
<td>0,025642213</td>
</tr>
</tbody>
</table>

Tab. 7.4 Results of calibration – Parameters (left) - Objective Function (right)
A check that can be made to check that the stoichiometric parameter values are reliable is as follows:

\[(Y_{H,STO} \times Y_{STO,S}) \times f_{STO} + (1 - f_{STO}) \times Y_{H,S}\]

This expression quantifies the COD balance in removal processes. This balance means that all the COD introduced, through the addition of acetate in the respirometric tests, must be found in the various biological processes at stoichiometric level. Therefore, this value will not be exactly 1 as part of the COD is lost, so it is deduced that the previous expression will have to give a value close to 1 to ensure that the values of the parameters make physical sense.

In this case, the formula gives as a result the value: 0.931685

This is consistent on what we said before.
Fig. 7.3 Observed OUR (grey line) and Simulated OUR with Calibrated Parameters (blue line) – Input Acetate 143 mgCOD/l

Tab. 7.6 Results of calibration – Parameters (left) - Objective Function (right)
A check that can be made to check that the stoichiometric parameter values are reliable is as follows:

\[
(Y_{H,STO} \times Y_{STO,S}) \times f_{STO} + (1 - f_{STO}) \times Y_{H,S}
\]

This expression quantifies the COD balance in removal processes. This balance means that all the COD introduced, through the addition of acetate in the respirometric tests, must be found in the various biological processes at stoichiometric level. Therefore, this value will not be exactly 1 as part of the COD is lost, so it is deduced that the previous expression will have to give a value close to 1 to ensure that the values of the parameters make physical sense.

In this case, the formula gives as a result the value: 0.900119712

This is consistent on what we said before.
Fig. 7.4 Observed OUR (brown line) and Simulated OUR with Calibrated Parameters (blue line) – Input Acetate 149 mgCOD/l

<table>
<thead>
<tr>
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<td>1,000</td>
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Tab. 7.8 Results of calibration – Parameters
A check that can be made to check that the stoichiometric parameter values are reliable is as follows:

\[(Y_{H,STO} \times Y_{STO,S}) \times f_{STO} + (1 - f_{STO}) \times Y_{H,S}\]

This expression quantifies the COD balance in removal processes. This balance means that all the COD introduced, through the addition of acetate in the respirometric tests, must be found in the various biological processes at stoichiometric level. Therefore, this value will not be exactly 1 as part of the COD is lost, so it is deduced that the previous expression will have to give a value close to 1 to ensure that the values of the parameters make physical sense.

In this case, the formula gives as a result the value: 0.925077366

This is consistent on what we said before.
7.3 **Calibration of Phase C Experimental Charts**

Initially, the Excel spreadsheet was set up, as indicated in paragraph 7.1.

Subsequently, the model was calibrated using the Excel "Solver" tool. This tool has been instructed to minimize the parameter "F" (Objective Function - specified in paragraph 7.1). This parameter preserves information on the discrepancy between the experimental observations and the simulated model. The fixed parameters that the Solver must vary have been specified, taking care to impose upper and lower limits that the latter can assume, in order to prevent the calibration method from finding values of the parameters that, even if the experimental data are well rented, do not make any physical sense.

GRG non-linear has been selected as the optimization method because all the methods proposed by the Solver converge on the same result.

The main problem, which did not bring the method to an adequate convergence, was found in the definition of the initial values to be given to the parameters. In fact, this method has a strong dependence on the initial values that are assigned to the terms to be optimized.

To overcome this problem, before the assignment of the Initial Values to be given to the parameters to be optimized, Sensitivity Analyses of these parameters were made, approaching as close as possible to the value that brings the simulated graph as close as possible to the one observed.

This procedure has been done for each stoichiometric and kinetic parameter of the model, after which, launching the Solver tool, excellent results have been obtained.
Fig. 7.5 Observed OUR (brown line) and Simulated OUR with Calibrated Parameters (blue line) – Input Acetate 232 mgCOD/l

Tab. 7.10 Results of calibration – Parameters

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14 March 2016
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*Tab. 7.11 Results of calibration - Objective Function*

Since the initial concentration of $X_H$ in previous calibrations was assumed on the basis of the initial sensitivity analysis, it was decided at this stage to include it in the parameters to be calibrated because no good solution was found from the sensitivity analysis.

A check that can be made to check that the stoichiometric parameter values are reliable is as follows:

\[
(Y_{H,STO} \times Y_{STO,S}) \times f_{STO} + (1 - f_{STO})\times Y_{H,S}
\]

This expression quantifies the COD balance in removal processes. This balance means that all the COD introduced, through the addition of acetate in the respirometric tests, must be found in the various biological processes at stoichiometric level. Therefore, this value will not be exactly 1 as part of the COD is lost, so it is deduced that the previous expression will have to give a value close to 1 to ensure that the values of the parameters make physical sense.

In this case, the formula gives as a result the value: 0.9382416

This is consistent on what we said before.
Fig. 7.6 Observed OUR (brown line) and Simulated OUR with Calibrated Parameters (blue line) – Input Acetate 285 mgCOD/l

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Tab. 7.12 Results of calibration – Parameters
Since the initial concentration of $X_H$ in previous calibrations was assumed on the basis of the initial sensitivity analysis, it was decided at this stage to include it in the parameters to be calibrated because no good solution was found from the sensitivity analysis.

A check that can be made to check that the stoichiometric parameter values are reliable is as follows:

$$\left(Y_{H,STO} \times Y_{STO,S}\right) \times f_{STO} + (1 - f_{STO}) \times Y_{H,S}$$

This expression quantifies the COD balance in removal processes. This balance means that all the COD introduced, through the addition of acetate in the respirometric tests, must be found in the various biological processes at stoichiometric level. Therefore, this value will not be exactly 1 as part of the COD is lost, so it is deduced that the previous expression will have to give a value close to 1 to ensure that the values of the parameters make physical sense.

In this case, the formula gives as a result the value: 0.986754

This is consistent on what we said before.
7.4 **CALIBRATION OF PHASE A EXPERIMENTAL CHARTS**

The graphs obtained from the experimental tests during this phase correspond to the behavior of bacteria in medium to low conditions of substrate concentration.

In addition, it should be considered that these are experimental observations measured at the beginning of this research, so they may be affected by errors, albeit small.

Initially, the Excel spreadsheet was set up, as indicated in paragraph 7.1.

Subsequently, the model was calibrated using the Excel "Solver" tool. This tool has been instructed to minimize the parameter "F" (Objective Function - specified in paragraph 7.1). This parameter preserves information on the discrepancy between the experimental observations and the simulated model. The fixed parameters that the Solver must vary have been specified, taking care to impose upper and lower limits that the latter can assume, in order to prevent the calibration method from finding values of the parameters that, even if the experimental data are well rented, do not make any physical sense.

GRG non-linear has been selected as the optimization method because all the methods proposed by the Solver converge on the same result.

The main problem, which did not bring the method to an adequate convergence, was found in the definition of the initial values to be given to the parameters. In fact, this method has a strong dependence on the initial values that are assigned to the terms to be optimized. To overcome this problem, before the assignment of the Initial Values to be given to the parameters to be optimized, Sensitivity Analyses of these parameters were made, approaching as close as possible to the value that brings the simulated graph as close as possible to the one observed. This procedure has been done for each stoichiometric and kinetic parameter of the model, after which, launching the Solver tool, excellent results have been obtained.
Fig. 7.7 Observed OUR (brown line) and Simulated OUR with Calibrated Parameters (blue line) – Input Acetate 54.703 mgCOD/l

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Tab. 7.14 Results of calibration – Parameters
Since the initial concentration of $X_H$ in previous calibrations was assumed on the basis of the initial sensitivity analysis, it was decided at this stage to include it in the parameters to be calibrated because no good solution was found from the sensitivity analysis.

A check that can be made to check that the stoichiometric parameter values are reliable is as follows:

$$\left(Y_{H,ST0} \times Y_{ST0,S}\right) \times f_{ST0} + \left(1 - f_{ST0}\right) \times Y_{H,S}$$

This expression quantifies the COD balance in removal processes. This balance means that all the COD introduced, through the addition of acetate in the respirometric tests, must be found in the various biological processes at stoichiometric level. Therefore, this value will not be exactly 1 as part of the COD is lost, so it is deduced that the previous expression will have to give a value close to 1 to ensure that the values of the parameters make physical sense.

In this case, the formula gives as a result the value: 0.904449085

This is consistent on what we said before.
Fig. 7.8 Observed OUR (brown line) and Simulated OUR with Calibrated Parameters (blue line) – Input Acetate 54.703 mgCOD/l

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Tab. 7.16 Results of calibration – Parameters
Since the initial concentration of $X_H$ in previous calibrations was assumed on the basis of the initial sensitivity analysis, it was decided at this stage to include it in the parameters to be calibrated because no good solution was found from the sensitivity analysis.

A check that can be made to check that the stoichiometric parameter values are reliable is as follows:

$$\left( Y_{H,STO} \times Y_{STO,S} \right) \times f_{STO} + (1 - f_{STO}) \times Y_{H,S}$$

This expression quantifies the COD balance in removal processes. This balance means that all the COD introduced, through the addition of acetate in the respirometric tests, must be found in the various biological processes at stoichiometric level. Therefore, this value will not be exactly 1 as part of the COD is lost, so it is deduced that the previous expression will have to give a value close to 1 to ensure that the values of the parameters make physical sense.

In this case, the formula gives as a result the value: 0.78742621

This is consistent on what we said before.
In the present thesis work, the graphs derived from experimental observations of respirometric tests have been calibrated.

In the previous chapter, the graphs designated for calibration are shown together with the results of the calibration.

The experimental tests, conducted to extrapolate the respirometric data, were developed over the years by feeding the beds of the Wetlands with different concentrations of COD mass load, this implies a subdivision of the OUR graphs according to the experimental "phase" in which they were extrapolated: phase A, phase C, phase D (details are given in chapter 6).

**Phase D**

The Phase D graphs in Chapter 7.2 all show a trend showing an initial peak due to the introduction of acetate into the reactor, an exponential increase in OUR values until a second global peak is reached, and a final downward trend until a stable regime of OUR values defined as the new endogenous level is reached. This new endogenous level is higher than the initial endogenous level because there has been a growth of bacteria.

As a result of the calibration, the values of the model parameters that best approximate the experimental observations were obtained. These values are reported in chapter 7 and show some agreement, especially with regard to stoichiometric parameters, which remain similar graphically.

This suggests a similar behavior of the bacterial flora in the various experimental tests and this is in accordance with the assumptions made. These assumptions predicted that the predominant phenomena that could be observed experimentally were those
of direct growth of heterotrophic bacteria on the substrate. This assumption derives from the way in which the beds are fed: greater amounts of COD mass load and greater concentrations of acetate in input influence the behavior of the bacteria, which, having much acetate available, get used to living in "Feast" conditions where the accumulation processes are not necessary for their survival. That's why, once the acetate in the system is finished, the graphs show a sharp descent with consequent stabilization at the final endogenous level.

Phase C

Of the graphs obtained at this stage, only 2 were chosen for calibration. This decision was suggested by the fact that they were the only ones to present a significant trend. The feeding of the beds in this phase was the one with the highest COD mass load and consequently, in the respirometric tests, higher acetate concentrations were used. These conditions favor more the flow of COD mass load towards the processes of direct growth on the substrate in an even more accentuated way than in the case of phase D.

Both graphs, also in this case, are calibrated with values of the parameters agreeing from the physical and biological point of view.

However, in these two cases the simulated graph is not perfectly adherent to the experimental observations, but this can be attributed to some systematic error during the tests or to biological micro-processes not considered because they are considered negligible.

Phase A

Of the graphs from the Phase A experimental tests, only two were considered suitable for calibration.
Phase A was the one with the lowest bed feeding and the lowest input acetate concentrations in respirometric tests.

Both calibrations, also in this case, show a certain agreement in the values of the parameters and with the assumptions made. In fact, with a lower concentration of COD mass load, bacteria get used to remain in "Famine" conditions and therefore develop the ability to accumulate COD, in the form of accumulation products, to be consumed once the substrate runs out. Therefore, the Storage on Substrate process is the predominant phenomenon.
9 References


Wallace, & Knight. (2006).
