

# **Analysis of the impact of the industrial fat, oil and grease (FOG) on a municipal wastewater treatment plant by means Respirometry and microscopic Bioindication**

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## **ABSTRACT**

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The main impact that the industrial fat, oil and grease (FOG) could make in a municipal wastewater treatment plant is mainly coming from its slowly biodegradable COD (sbCOD)

The wastewater entering in a municipal WWTP tends to include a percentage of slowly biodegradable COD in the range of 35 to 65% of the total COD; but when this percentage surpasses the habitual range, normally it is due to the presence of any industrial waste that can adversely affect the process and which is usually called recalcitrant COD.

Just in the case of a high slowly biodegradable COD coming from FOG wastes, the process become into a specific bulking and foaming phenomena accompanied by specific filamentous bacteria, a deteriorated sludge under the state of dispersed flocculation and certain degree of bioactivity reduction.

Often those effects are erroneously classified as toxicity, when in fact chances are it is not.

BM Respirometry and microscopic Bioindication stand as an effective combination to analyze this situation accurately and figure out some conclusions that could afford to take the corrective actions that can reduce the harmful effects coming from this situation.

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### **Key words**

*Respirometry, Bioindication, slowly biodegradable COD, readily biodegradable COD, recalcitrant, activated sludge,*

## 1. Introduction

The hydrolysis process of a high percentage of slowly biodegradable COD in the total COD has an important impact in the biomass and, simply said, this is mainly coming from the accused impoverishment of the food (organic matter) quality for the microorganisms.

It must taken into account that the bacteria responsible of the substrate removal are in need of the soluble readily biodegradable COD for their growth and, when the COD profile includes the presence of a high slowly biodegradable COD fraction, they may not reach to satisfy their metabolic needs; in this way, its reproduction and flocculation abilities are significantly depleted.

In the case the treatment plant includes the biological process of nitrification-denitrification, injury tends to be more accused in the anoxic denitrification process where most probable the concentration of readily biodegradable COD may be too small for its normal performance.

As we had mentioned, one of the most effective methods to analyze this complex situation is by means the Respirometry and microscopic Bioindication. However, is also important to stress that the effectiveness of the respirometric tests, results and conclusions will largely depend on the type of respirometry in use, since the respirometer must have the ability to accurately determine the stoichiometric yield coefficient, automatic measurements of the COD biodegradable fractions within relatively short time, as well as the specific COD utilization rate.

This paper includes the description of a protocol based on a set of respirometric tests which can be performed within a relative short time and that can figure out the concentration of any slowly biodegradable COD and the its consequences in the activated sludge. It will also include a real case of study composed by the Respirometry followed by the Bioindication part where somehow complemented and confirmed some of the most important conclusions

## 2. Respirometry

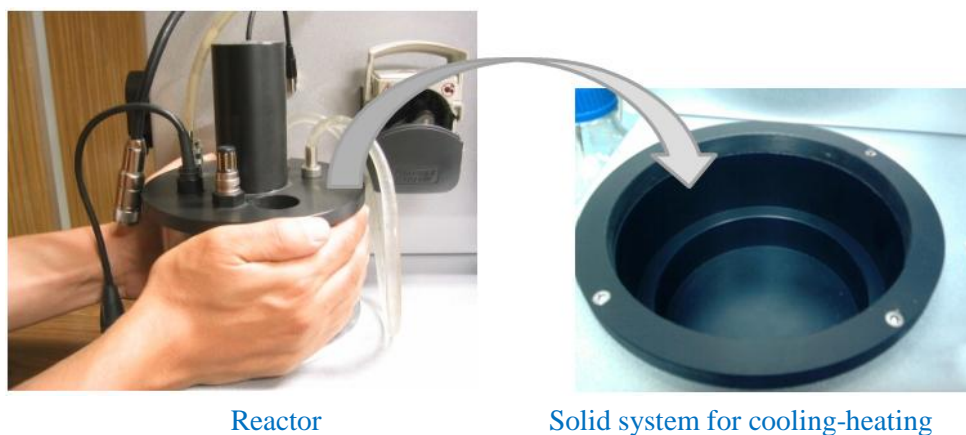
### 2.1. BM Respirometer

As above discussed, this kind of situation can be carried out by means of the BM Respirometry.

The line of analyzers that are making use of the BM Respirometry, manufactured by Surcis, S.L. are unique systems that possesses the ability to be set for different conditions of pH, Temperature, Oxygen, Relationship sample / sludge and others (before and during the test performance)

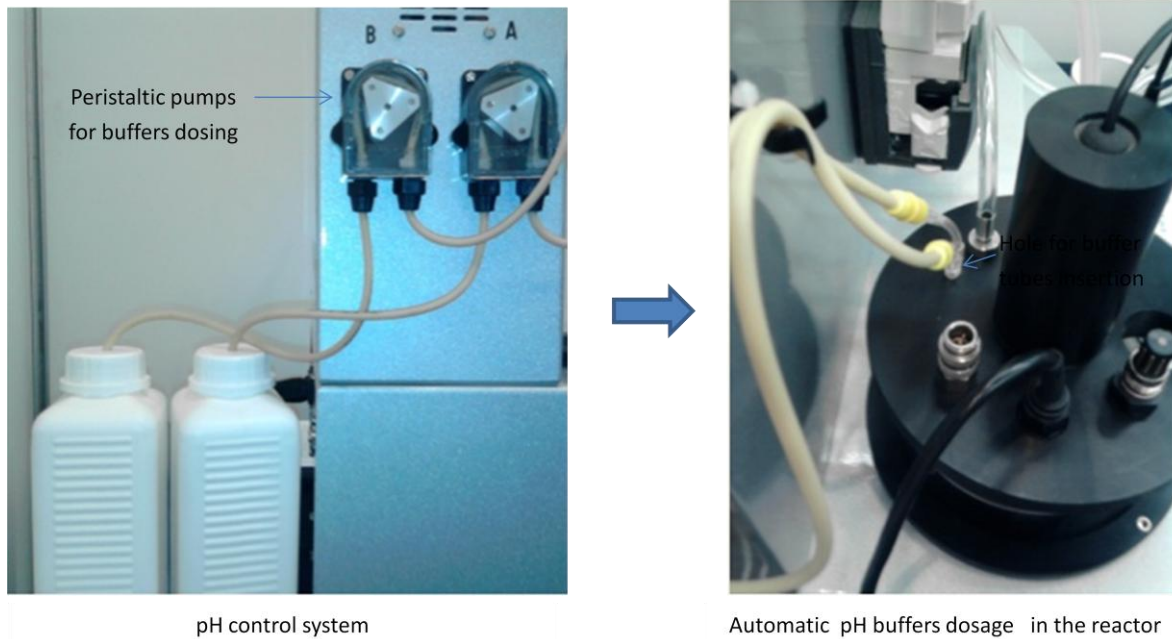
Within the important features of the BM Respirometers we can highlight the automatic cooling-heating system (peltier & heater) integrated in the own analyzer console (**Figure 1**)

**Figure 1.** Settlement of the reactor in the automatic cooling-heating system



BM-Advance also includes a pH monitoring and control system, with the ability to set a pH for the test performance and change its value any time during the test (**Figure 2**)

**Figure 2.** Automatic system for pH control



BM-Advance Pro model, besides oxygen and pH sensors, also includes an ORP sensor.

Tests are configured from a common settings board (**Figure 3**) where we can set all data needed for automatic calculations as well as conditions: Temperature, pH, Oxygen, Aeration, Recirculation rates.

**Figure 3.** Common settings board for any test in a BM respirometer

New Test

Test type:  
R  
OUR  
Cyclic OUR

Name: New Test  
Operator:  
Filename: Search  
Data interval: 2 s.

Vf: 1000,00 ml Solids: 2,60 g/L CO: 126,05  
Vm: 50,00 ml Y: 0,67 DO Low: 2,0  
fd: Auto 21 Readings < 0 DO High: 6,0  
Force Cb: 0,00

Board control settings during test

Temperature control: 20,00  
PH Control: 7,00 Hysteresis: 0,20

Peristaltic pump: 2  
Aeration: 55

Cancel Accept

In this setting board, we can select three different operation modes: OUR, Cyclic OUR and R (**Table 1**) Each of those modes includes a specific package of measurements to apply in the corresponding application to develop.

**Table 1.** Operation modes and automatic measurements in the BM Respirometry

Test types	Automatic measurements	Description
OUR	OUR (mg O <sub>2</sub> /L.h)	Oxygen uptake rate
	SOUR (mg O <sub>2</sub> /VSS.h)	Specific OUR
OUR cíclico	OUR (mg O <sub>2</sub> /L.h)	Chain of sequential OUR measurements
	SOUR (mg O <sub>2</sub> /VSS.h)	Chain of sequential SOUR measurements
R	Rs (mg O <sub>2</sub> /L.h)	Continuous measurement of the exogenous respiration rate under uninterrupted aeration
	CO (mg O <sub>2</sub> /L)	Consummed oxygen from substrate oxidation
	bCOD (mg/L)	Biodegradable COD (soluble or total)
	q <sub>H</sub> (mg DQO/mgSS.d)	Soecific substrate utilization rate
	U (mg DQO/L.h)	Substrate utilization rate

## 2.2. Heterotrophic yield coefficient

### 2.2. The importance of the stoichiometric coefficient $Y_H$

This coefficient, when referred to the oxygen demand, represents the percentage (expressed as per unit) of soluble COD destined to the heterotrophic biomass growing.

The  $Y_H$  analysis, such as we have explained in the operation protocol, represents a critical stoichiometric parameter when assessing the biomass health in any activated sludge through its growing capacity.

BM Respirometry determines the  $Y_H$  coefficient from the automatic measurements of the consumed oxygen (CO) of a soluble substrate from which the COD value is already known.

### 2.3. Operation protocol

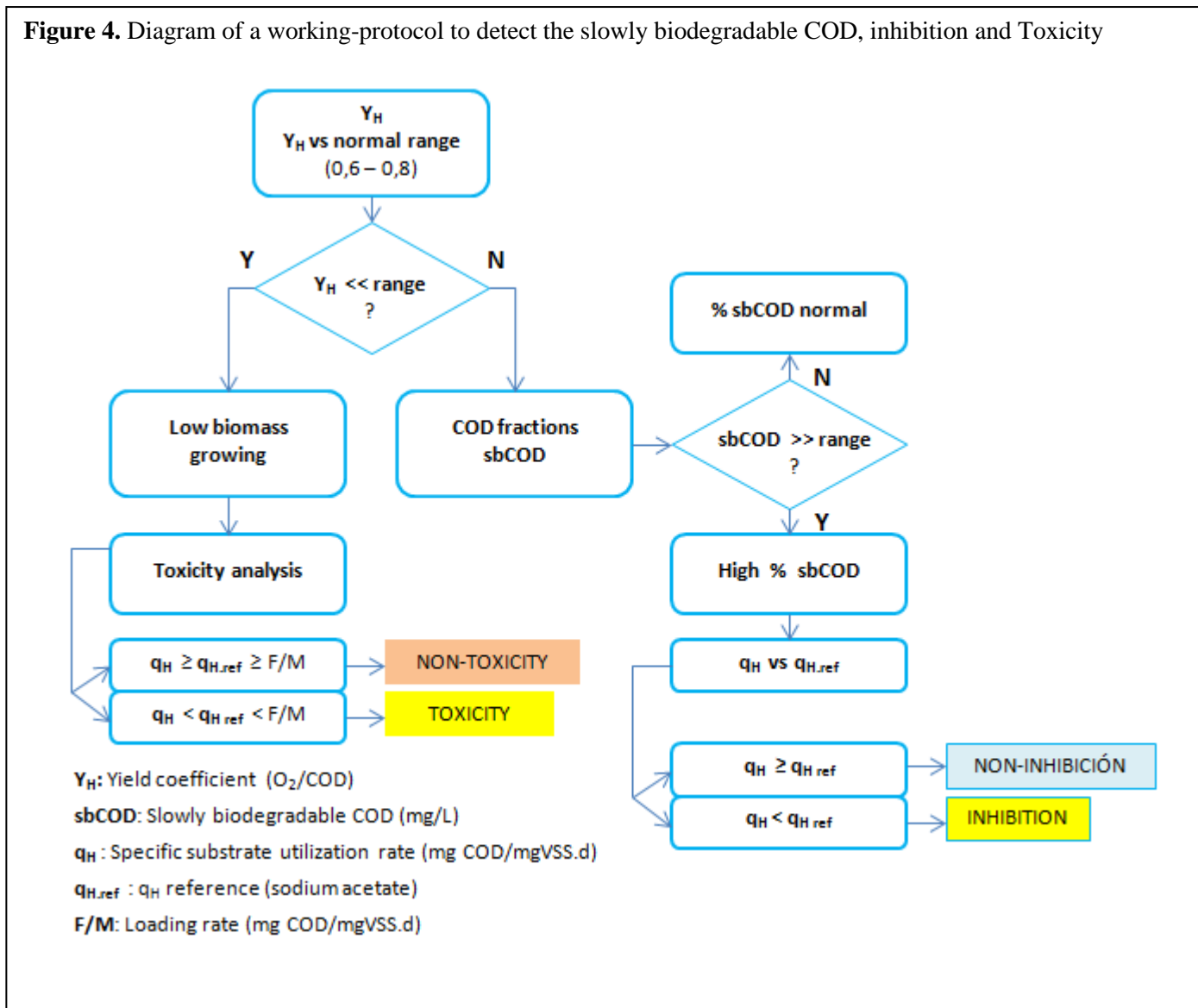
Depending on the possible variants of the case to analyze, it can be several possibilities to make out an operation protocol. Here, we have tilted toward a non complicated basic protocol that practically can be carried out with no more than three or four relatively short respirometric tests (**Figure 4**).

This protocol starts with the heterotrophic yield determination  $Y_H$ . Once determined, it is assessed by comparing its value with a normal range (0,6 ~ 0,8) In this way, when the heterotrophic biomass is fed with the soluble substrate that we had used as reference, we can discern if it is under a normal yield or not.

In case the  $Y_H$  is significantly lower than the minimum value of its normal range, we can assure that the biomass growing can be also low. In this case, we have to confirm if the process is under the effects of any toxicity or not and, on this purpose, the protocol goes to a procedure based in the substrate utilization rate ( $q_H$ ) compared with the substrate utilization rate from the reference compound ( $q_{H.ref}$ ).



**Figure 4.** Diagram of a working-protocol to detect the slowly biodegradable COD, inhibition and Toxicity



When  $Y_H$  is in range, independently of the physical state of the sludge, the protocol can go to the determination of the representative COD fractions: bCOD (degradable COD), rbCOD (readily biodegradable COD), sbCOD (slowly biodegradable COD) and iCOD (inert or non-biodegradable COD)

Then, we will analyze the percentage of sbCOD to know if it is higher than the top limit of its normal range. If so, we will assess the biological activity as we did before: by analyzing the substrate utilization rate ( $q_H$ ) to be compared with a reference substrate utilization rate ( $q_{H.ref}$ )

In case  $q_H$  is equal or higher than  $q_{H.ref}$ , we can confirm that there is not any inhibition in the process; but, if  $q_H$  is lower than  $q_{H.ref}$ , then it would be a clear case of inhibition.

It is important to remark that in this protocol the Toxicity and Inhibition are considered as two different items: Toxicity always includes Inhibition; but Inhibition could not include Toxicity (going towards one or the other will depend of the  $Y_H$  value).

### 3. Case of study: high percentage of slowly biodegradable COD and activated sludge deterioration

This study comes from the series of works which SURCIS Company regularly conducts within the annual inter-laboratory program organized by GBS research group in Spain.

This is a case where the presence of a slowly biodegradable COD will be perfectly detected and, because of its specific nature (FOG) and the damage infringed to the normal process of flocculation, it can be classified as recalcitrant.

The most representative of this process of activated sludge data outlined in the following table:

**Table 2.** Representative data of the study

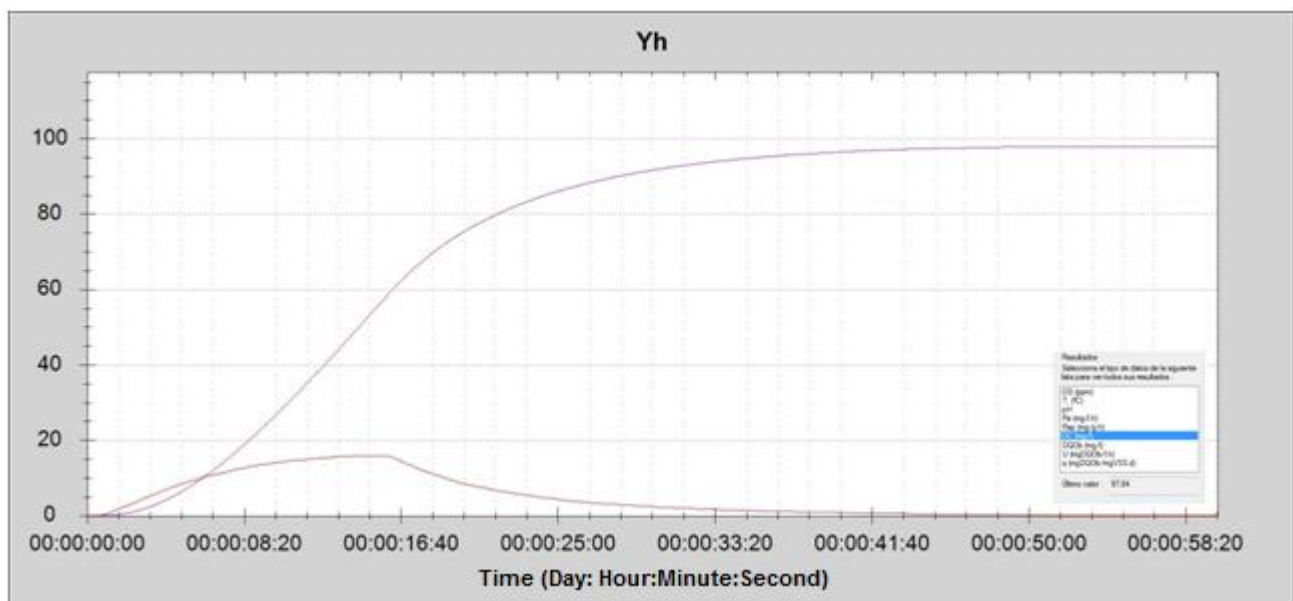
Parameter	Description & Average value
Process type	4 parallel aeration basins with turbines. Plug-flow type
Nitrification	No
Anoxic zone	37% - only for agitation to avoid sedimentation
Temperature	15 °C
COD in influent wastewater	444 mg/L
MLSS / MLVSS	1800 mg/L / 1422 mg/L
SRT	5 d
SVI	160 mg/l
Bulk dissolved oxygen	3 ppm
Fat, Oils, Grease (FOG)	Yes
Foaming	Yes – Brown color

### 3.1. Respirometry

#### 3.1.1. Yield coefficient of the heterotrophic biomass ( $Y_H$ )

The  $Y_H$  is determined in one single respirometric test (**Figure 5**) by making use of a standard solution of sodium acetate with a COD of 300 mg/l ( $COD_{ac}$ ) and endogenous sludge free of any other substrate. In our particular case it was used recirculation activated sludge (RAS) under endogenous state.

**Figure 5.** Simultaneous respirograms of exogenous respiration rate ( $R_s$ ) and consumed oxygen (CO) for  $Y_H$  determination



**Table 3.** Results of parameters for  $Y_H$  calculation

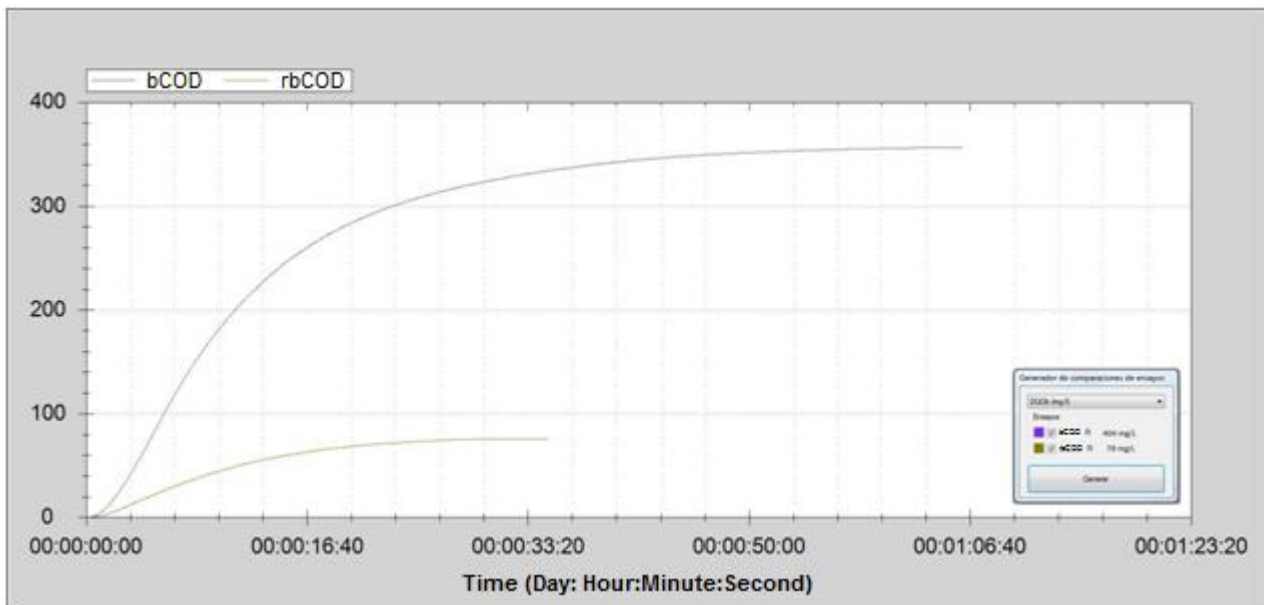
Parameter	Description	Calculation	Value
CO	Consumed oxygen	Automatic	98 mg/L
$Y_H$	Yield coefficient	$1 - CO/COD_{ac}$	0.67 mg $O_2$ /mg COD

The  $Y_H$  (**Table 3**) is within a normal range (0.6 – 0.8). That means the biomass has a normal yield and confirms the active biomass is not affected by any kind of toxicity.

### 3.1.2. COD fractions

We determine the biodegradable COD (bCOD) and readily biodegradable COD (rbCOD) by means two respirometric R tests. For bCOD it was used endogenous sludge and influent wastewater and for rbCOD, endogenous sludge and truly soluble wastewater (**Figure 6**) From both results, by also making use of the total COD, the rest of the most representative COD fractions values and their percentages in the total COD are then calculated (**Table 4**)

**Figure 6.** Overlaid bCOD and rbCOD respirograms from their respective respirometric tests.



**Table 4.** COD fractions

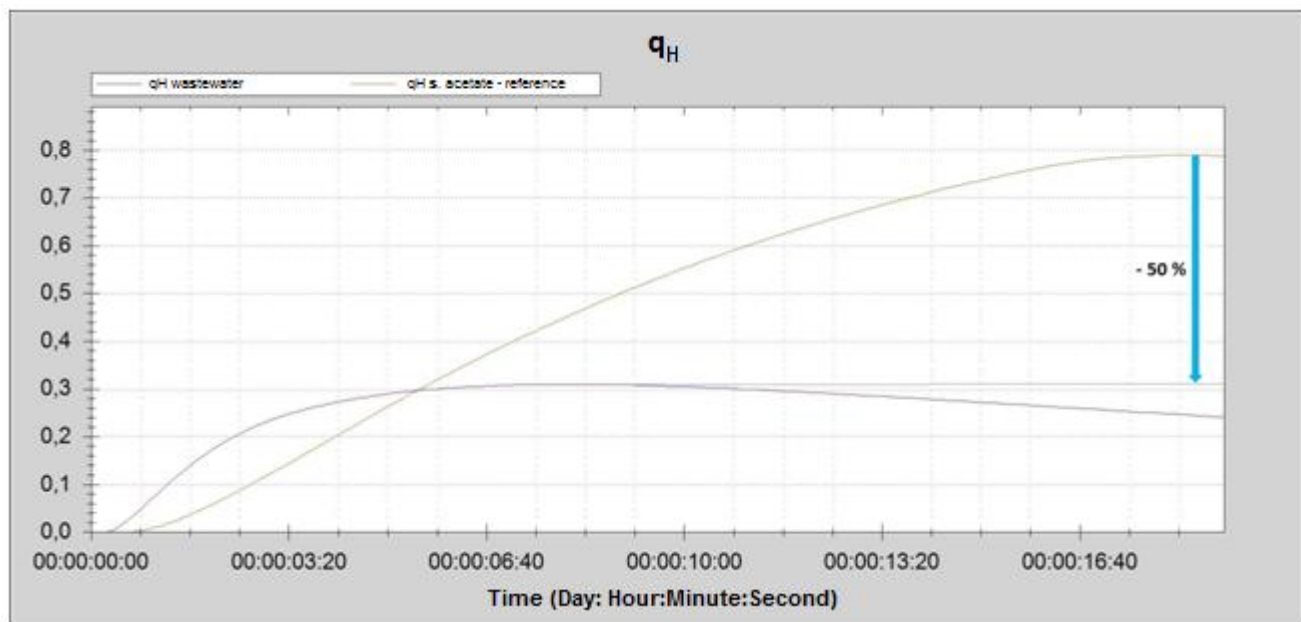
COD fraction	Description	Value	% in total COD
bCOD	Biodegradable COD	404 mg/L	91 %
rbCOD	Readily biodegradable COD	79 mg/L	17 %
sbCOD	Slowly biodegradable COD	325 mg/L	74 %
nbCOD	Non-biodegradable COD	40 mg/L	9 %

From the COD fractions results, it is important to highlight the value of 74 % of sbCOD which stays beyond the usual range in urban wastewater (40 – 65 %) In this case, this high percentage of slowly biodegradable COD in all likelihood is coming from the presence of fat, oil and grease (FOG) specified in the data of the process (**Table 2**)

### 3.1.3. Comparison of the maximum specific substrate utilization rate between the wastewater and a standard compound as a reference

Following the working protocol diagram (**Figure 4**), the BM respirometer can now automatically determine two specific substrate utilization rate: one from wastewater ( $q_H$ ) and the other from the reference substrate ( $q_{H,ref}$ ), both with identical loading rate.

**Figure 7.** Overlaid  $q_H$  respirograms from wastewater and sodium acetate (reference) tests



In the maximum value of the specific substrate utilization rate ( $q_H$ ) overlaid respirograms it is observed a decrease of 50% from wastewater versus the value of the reference (standard compound: sodium acetate). For this reason, we can confirm that the cause of low activity and slow COD degradation comes from wastewater nature and not from the activated sludge biomass state. Likewise, it follows that the low biological activity that the activated sludge develops in the wastewater is due to the high percentage of sbCOD which undoubtedly comes from the high concentration of FOG.

From the plant data it is known that the sludge is presenting a poor floc structure. However, the  $q_H$  tests are demonstrating that the biomass can develop a good biological activity with any other substrate different from wastewater and that it has potential for recovery.

### 3.1.4. Conclusions from the respirometric study

1. The normal  $Y_H$  (0.67) and the good response of the activated sludge to the reference standard compound (sodium acetate) confirm that the sludge is not under any toxic effect.
2. The COD fractions tests have detected a very high slowly biodegradable COD that because its specific nature (FOG) could be classified as recalcitrant COD.
3. When comparing the specific substrate utilization rate of the wastewater in the sludge versus the one from a standard compound, it was detected and inhibition of 50%. Since this effect is only coming from the wastewater nature and not affecting the vital constants of the microorganisms in the activated sludge, this inhibition cannot be considered as a lethal toxicity.
4. In summary, it is assumed that the causes of the bioactivity in the sludge and foaming presence are based on the following points:
  - High percentage of slowly biodegradable COD of recalcitrant nature.
  - Relatively low percentage of soluble readily biodegradable COD.
  - The relatively low percentage of readily biodegradable COD is resulting in a low soluble biodegradable COD loading rate which leads to a certain lack of food in the microorganism.
  - On the current conditions, it is logically assumed that the anoxic zone for agitation (specified in the process data) is seriously hampering the biomass oxygenation.

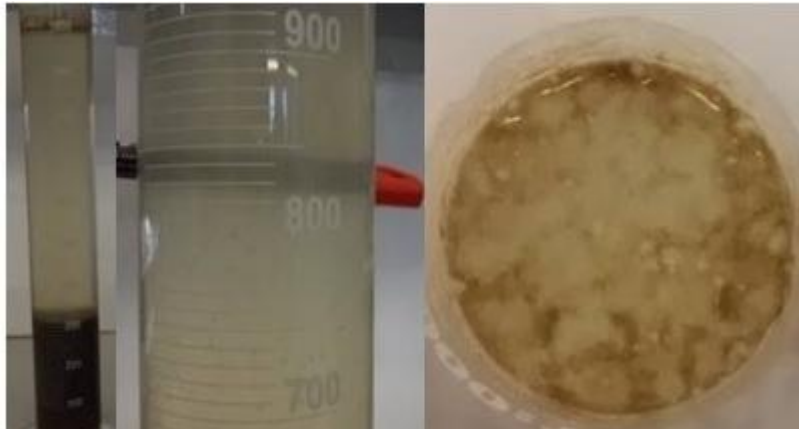


## 3.2. Microscopic Bioindication

### 3.2.1. Microscopic assessment

From a general microscopic Bioindication assessment, the sludge could be classified as mediocre. It has settling problems, some white foaming and a thin waxy layer on surface (**Figure 8**)

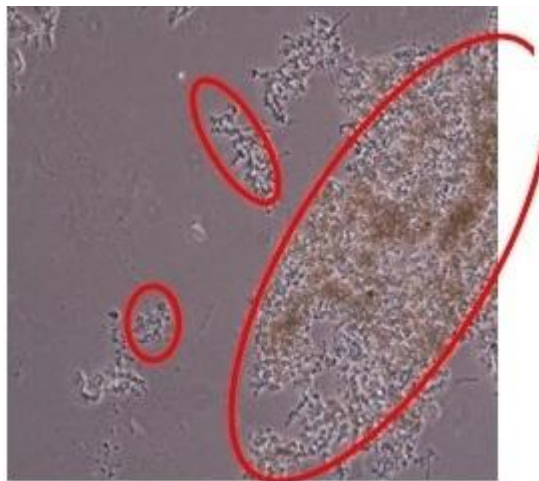
**Figura 8.** Microscopic assessment of the sludge after 30 minutes: Some poor sludge settling, high turbidity, white foaming and thin waxy layer on surface.



With the microscopic observation, it is noted that the high amount of free bacteria and the presence of dispersed micro-flocs are the cause of high turbidity.

These micro-flocs are of very different sizes (**Figure 9**), and this diversity boosts the negative effects stemming from the presence of filamentous bacteria in the activated sludge such as its disintegration and poor compaction.

**Figura 9.** Assessment of the microscopic features of the sludge: Size of the floccules.

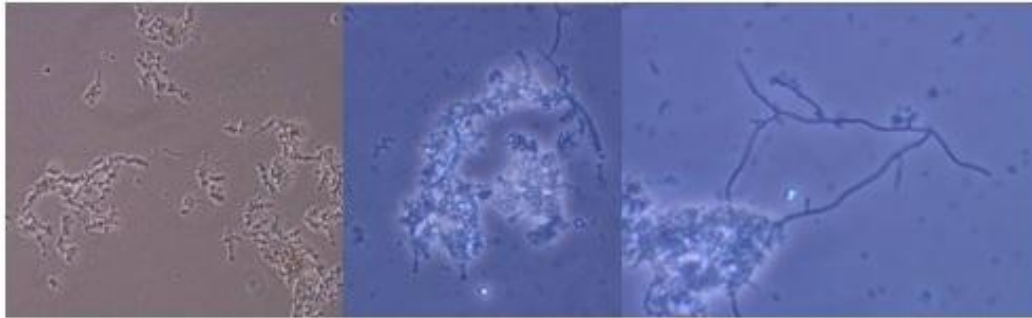


### 3.2.2. Biotic component:

Filamentous bacteria: The dominance of Nocardiforms (**Figure 10**) defines a situation of controlled foaming. Among the secondary filamentous it has been identified the *Nostocoida limicola* and morphotype 021N. Other microorganisms detected in lower concentration have been the following: T1701, *Thiothrix*, T1851, *Streptococcus*, *Haliscomenobacter hydrossis*, Hongos, *Beggiatoa*, T0041/0675, *Flexibacter* y T0961.

In general, the community of filamentous microorganisms share a common development guidelines that are closely linked to nutritional deficiency, limited oxygen, and especially to low loading rates (coincident with the respirometric analysis)

**Figure 10.** *Nocardiforms* (Majority filamentous), *In vivo*, phase contrast, 100x, 200x y 1000x.

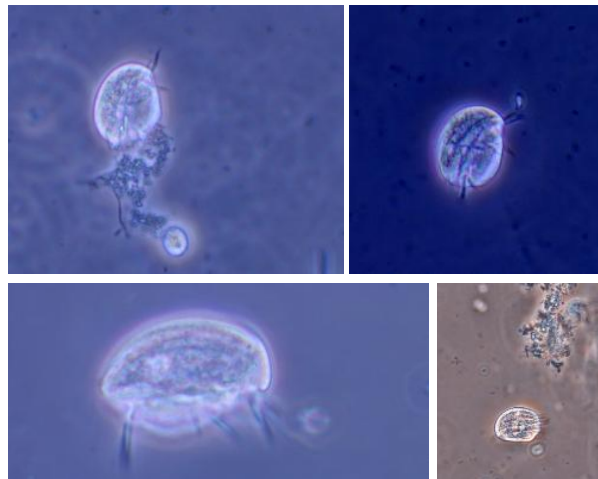


Although they also appear some fragments in the inter-floccular spaces, the development of filamentous bacteria is mainly associated to the own floc. These species are found in the relative abundance of 4 (5-20 filamentous per floc) that, despite not being very high, the predominant role of the Nocardiforms generates the floccular disintegration.

### 3.2.3. Protists

The dominant group in this activated sludge is the crawling bacterivorous *Aspidisca cicada* (**Figure 11**) as the single representative. Organism that develops in a wide range of environmental conditions.

**Figure 11.** *Aspidisca cicada*, *In vivo*, phase contrast, 100x, 200x y 400x.



### 3.2.3. Conclusions from the Bioindication study

1. There is a clear problem of deflocculation. This problem is due to the presence of filamentous bacteria in a process where, thanks to some strict operational controls, it can get however an effluent profile within the permitted limitations.
2. According to the data of the plant and respirometric confirmation, there is an important concentration of slowly biodegradable COD coming from fat, oil and grease (FOG), which conducts to a situation of nutritional deficiency.

#### 4. Proposed solutions to the current situation

The presence of FOG with its slowly biodegradable COD of recalcitrant nature brings the logical consequence of a low loading rate of the effective biodegradable soluble COD. In fact, both the COD fractioning from respirometry and the community of filamentous organisms detected in the bioindication are demonstrating this situation.

In simple words, the micro-organisms are lacking good food and to remedy this situation, the following points are proposed:

1. If possible, reduce or cut the FOG waste in the wastewater influent which is the direct cause of the high concentration of slowly biodegradable COD.
2. Temporarily increase the global COD loading rate (F/M) by gradually lowering the sludge retention time (SRT). This can result in a temporary reduction of the process performance; but this measure is actually to recover the health of the microorganisms; and, as soon as this happens, the process can return to their normal operating parameters.

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