The use of Respirometry as a tool for the diagnosis of Waste Water Treatment Plants. A real case study in Southern Spain.

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Abstract

The influence of operating conditions of wastewater treatment plants in microbial composition of activated sludge should be controlled to evaluate the efficiency of the plant. Respirometry has been widely applied as a useful tool for a better understanding of the processes taking place in activated sludge system. In the present study, respirometry tests have provided relevant information about possible faults in the plant and potential improvements. With this aim, experimental tests were applied to a real wastewater treatment plant serving an equivalent population of 730,000. To evaluate the sludge activated process, the samples of mixed liquor studied were: those collected from inlet and outlet flow of reactor system and the laboratory prepared samples from mixing those from the influent/effluent wastewater to/from reactor with the returned activated sludge. The comparison of reference values with experimental results of factor charge (0.9-1.5), organic loading rate (>0.5 $g_{BOD}\ g_{MLVSS}^{-1}\ d^{-1}$) and specific oxygen uptake rate in mixed liquor for outlet samples from reactor (mainly > 10 $mg_{O2}\ g^{-1}\ h^{-1}$) obtained from respirometry tests revealed that the plant was operated at low efficiency and at overload. These conditions have a direct influence on the efficiency of biological treatment and the secondary clarifier operation. The sedimentation of activated sludge was evaluated through the determination of the sludge volume index during 10 months obtaining an average value of 208 mL g^{-1}. Respirometry tests also revealed that the decrease of biomass activity was associated with the wastewater instead of with the biomass.

Keywords:

Respirometry, WWTP optimization, WWTP diagnosis

Highlights

- Respirometry as a tool for detecting problems of activated sludge system.
- Detection of operation at low efficiency and at overloads conditions of a real WWTP.
- The reduction of biomass activity was associated with the wastewater.

Nomenclature

b_H	Decay rate of heterotrophic biomass, d^{-1}
BOD	Biochemical Oxygen Demand, $mg_{O2}\ L^{-1}$
CO	Consumed oxygen in the wastewater, $mg_{O2}\ L^{-1}$
COD	Chemical Oxygen Demand, $mg_{O2}\ L^{-1}$
COD_e	COD removed in the biological reactor, $mg_{\ O2}\ L^{-1}$
COD_S	Soluble COD, $mg_{O2}\ L^{-1}$
DO	Dissolved Oxygen, $mg\ L^{-1}$
f_{cv}	COD to VSS ratio $mg_{CODbact.}\ mg_{MLVSS}^{-1}$
FC	Factor Charge
F/M	Organic loading rate, Food to Microorganism ratio, $kg_{BOD}\cdot kg_{MLVSS}^{-1}\ d^{-1}$
HRT	Hydraulic Retention Time, h
k:	First-order reaction rate constant of COD consume, d^{-1}
k_d	Biomass fraction oxidized per day during endogenous respiration, d^{-1}
K_{DO}	Half-saturation constant for dissolved oxygen, $mg\ L^{-1}$
K_S	Half-saturation constant for dissolved substrate, $mg\ L^{-1}$
MLSS	Mixed Liquor Suspended Solids, $mg\ L^{-1}$
MLVSS	Mixed Liquor Volatile Suspended Solids, $mg\ L^{-1}$
OUR	Oxygen Uptake Rate in mixed liquor, $mg_{O2}\ L^{-1}\ h^{-1}$
OUR_{end}	Endogenous respiration rate, $mg_{O2}\ L^{-1}\ h^{-1}$
$OUR_{end.A}$	Endogenous respiration rate of autotrophic bacteria, $mg_{O2}\ L^{-1}\ h^{-1}$
$OUR_{end.H}$	Endogenous respiration rate of heterotrophic bacteria, $mg_{O2}\ L^{-1}\ h^{-1}$
OUR_N	Oxygen uptake rate in mixed liquor by nitrification, $mg_{O2}\ L^{-1}\ h^{-1}$
OUR_S	Oxygen uptake rate for organic substrate oxidation , $mg_{O2}\ L^{-1}\ h^{-1}$
q	Specific COD utilization rate, $mg_{COD}\ mg_{MLVSS}^{-1}\ d^{-1}$

q_{DO}	Specific COD utilization rate under DO influence, $mg_{COD}\ mg_{MLVSS}^{-1}\ d^{-1}$
R	Recirculation Rate
R_S	Actual dynamic respiration rate, $mg_{O_2}\ L^{-1}\ h^{-1}$
S	COD of dissolved substrate in the biological reactor, $mg\ L^{-1}$
S_{ef}	Effluent soluble COD from the biological reactor $mg\ L^{-1}$
S_{in}	Influent soluble COD to the biological reactor $mg\ L^{-1}$
SOUR	Specific Oxygen Uptake Rate in mixed liquor, $mg_{O_2}\ g^{-1}\ h^{-1}$
SRT	Solid Retention Time, d
SVI	Sludge Volume Index, mLg^{-1},
U	Maxima COD utilization rate, $mg_{COD}\ L^{-1}\ h^{-1}$
U_{act}	Real COD utilization rate in the biological process, $mg_{COD}\ L^{-1}\ d^{-1}$
X_V	Biomass concentration in the biological reactor, $mg_{MLVSS}\ L^{-1}$
X_H	Active heterotrophic biomass concentration, $mg_{COD}\ L^{-1}$
$Y_{H.COD}$	Yield coefficient of heterotrophic biomass referred to chemical oxygen demand, $mg_{CODbact}\ mg_{CODs}^{-1}$
$Y_{H.VSS}$	Yield coefficient of heterotrophic bacteria expressed as substrate converted to biomass $mg_{MLVSS}\ mg_{CODs}^{-1}$
Y_{obs}	Observed heterotrophic cellular yield coefficient $mg_{MLVSS}\ mg_{CODs}^{-1}$
μ_H	Growing rate of the heterotrophic biomass, d^{-1}
$\mu_{H\max}$	Maxima growing rate of the heterotrophic biomass, d^{-1}

1. Introduction

The activated sludge (AS) process, based on the production of a high-quality effluent using microorganism to degrade organic contaminants contained in wastewater, is the most widely used biological process [1]. The AS processes enhance the biodegradation as a results of the large number of microbes metabolizing the food source [2]. The operation could be divided mainly into three steps: a) sorption, the food source is available for the microorganisms; b) growth, the soluble and suspended organic matter is metabolized by microorganism; c) settling, the flocculate biomass is separated from the suspension to form sludge by gravity and the final effluent, theoretically free of solids [3]. The AS experimental system consists basically of the aeration tank and a settlement tank, known as the clarifier. First, wastewater from the primary treatment is added to the aeration tank containing the microorganisms. The aeration in AS process aims not only at supplying oxygen in the reactor for microorganism respiration but also at ensuring maximum contact between the surface of the microorganisms and the wastewater by agitation. It should be noted that the AS capacity to flocculate makes economically viable to be applied in large scale installations due to the acceptable sedimentation rates of flocs in clarifiers. Recirculation of AS from the clarifier into the aeration tank after settling is usually carried out to ensure the required microbial population [4].

The variation of influencing factors in wastewater treatment plants (WWTPs) causes important changes into the microbial composition of AS. Thus, it is required to develop a procedure to control the interaction of biomass with wastewater components. Respirometry, as a tool for modelling and optimizing the AS process, provides useful information associated with substrate removal and biomass growth. It should be noted that the processes requiring oxygen are, mainly: carbonaceous material removal from waste stream by the growth of heterotrophic bacteria and nitrification and oxidation of ammonia nitrogen to nitrate-nitrogen by autotrophic organism [5].

The endogenous phase plays an important role in the respirometric methods. During this phase, the loss of microbial activity takes place as a result of no external substrate is added. Thus, the internal generation of substrate is associated with decay and hydrolysis processes. The measure of endogenous respiration rate allows through evaluation of respirograms the determination of the biochemical oxygen demands and AS growth kinetics [6,7].

The International Association on Water Quality (IAWQ) deals with the application of respirometry in control of the AS process. In 1998, the principles of respiration rate measurement and respirometry-based control strategies were published [8]. IAWQ developed the Activated Sludge Models (ASM) describing biological and chemical processes taking place in AS system. In 1987, it was published the ASM1 which describes biological oxidation of carbon, nitrification and denitrification processes [9]. This widely-applied model presents some limitation about phosphorus removal entailing the development of ASM2 in 1995 [10] and ASM2d in 1999 [11]. The later development of ASM3 coped with limitations of previous models such as difference of decay rates of nitrifiers under aerobic and anoxic conditions [12]. Some authors have modified and calibrated the ASM3 model with full-scale operating data to describe the performance of a real WWTP [13].

In addition to the use of respirometry in control, it is applied to quantify and to detect disturbances that could entail a poor performance of the plant. For example, the presence of toxicants could involve the plant objectives were not reached [1]. Standardized test widely used along the time are based on the reduction of respiration rate of AS in presence of toxicants [14–16]. Currently, there is a wide range of respirometers from manually operated bottle containing a dissolved oxygen sensor to sophisticate automatic instrument. However, the operation of all respirometers is based on the evaluation of the rate at which biomass takes up dissolved oxygen from the liquid, the parameter widely known as Oxygen Uptaken Rate (OUR) [5]. Examples of the wide-range of relevant information type provided are: direct indication of activity of biomass, characteristics of wastewater as concentration, fractionation and toxicity and quantitative information about the way of interaction of biomass with wastewater components [8].

The potential of respirometry has been widely probed in numerous applications. Vasiliadou et al. [17] carried out AS respirometry tests to study the negative effects of pharmaceutical compounds on the performance of secondary biological process in WWTP. The applied methodology allowed them to obtain interesting results about the toxic and inhibitory effects of pharmaceuticals on microbial communities of AS. Mrafkova et al. [18] evaluated the influence of heavy metals presence in AS by respirometric measurements using an oxygen probe. They concluded that the measure of OUR plays an important role on the identification of possible inhibitory effects of heavy metals present in aqueous fraction. Ning et al. [19] dealt with the control of nitrogen nutrient addition to AS process in WWTPs, a critical parameter in biological treatments. With this aim, they studied two AS samples using respirometric methods: a municipal sludge and an industrial sludge. From results, experimental methods were developed to quantify and identify nitrogen nutrient deficiency in AS. Oliveira et al. [20] calculated the heterotrophic kinetic parameters in AS plant modified by placing a sludge retention reactor through respirometry. In this work, it was compared two different plant configurations to evaluate the minimization of excess sludge. Also, the respirometry tests are used to diagnosis and optimization of WWTPs, Pons et al. [21] proposed a method based on the evaluation of OUR curve to improve the detection of faults in a real plant. They concluded that changes in characteristic of incoming wastewater could be detected by respirometry tests.

To the best of our knowledge, there is a clear lack of studies dealing with the use of respirometry as a tool for monitoring and optimizing the AS processes in full-scale WWTPs. Therefore, the present study proposes a methodology based on the combination of respirometry tests with conventional procedures to evaluate a real WWTP. From experimental results, it was obtained relevant conclusions regarding possible faults in the plant operation and potential improvements.

2. Material and methods

2.1. Wastewater Treatment Plant

This study was carried out in the WWTP of Guadalhorce located in Malaga and operated by the Malaga Municipal Water Company (EMASA). The plant, schematically shown in Fig. 1, treats about 165,000 m^3 d^{-1} serving an equivalent population of 730,000. The plant is designed to treat a municipal effluent load of 1,280,000 population equivalent.

The wastewater is subjected to preliminary treatment, primary decantation, activated sludge processes as secondary treatment and tertiary treatment. The sewage sludge is stabilized by mesophilic anaerobic digestion followed by mechanical and thermal dewatering.

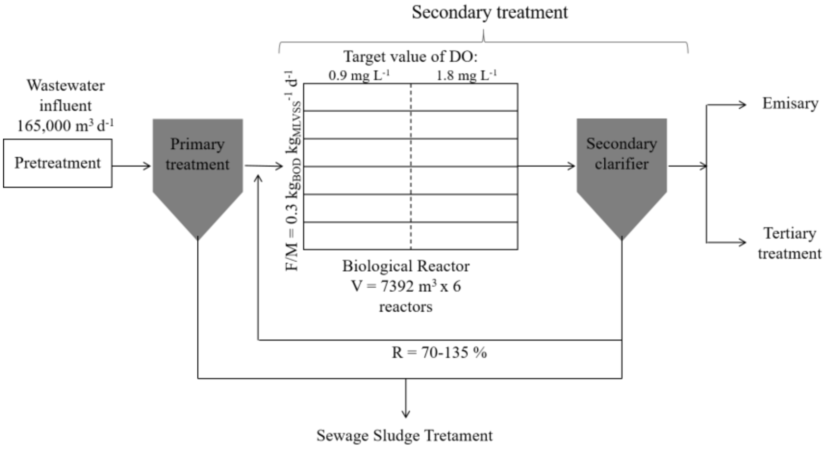

Figure 1. Schematic diagram of the Guadalhorce WWTP.

The biological wastewater treatment consist of 6 rectangular reactors (Fig. 2), with a volume of 7392 m^3 of which 3 or 4 units are usually in operation. The aeration system supplies the required oxygen to meet a concentration of dissolved oxygen (DO) value of 0.9 mg L^{-1} from the inlet to the middle of the reactor and a concentration value of 1.8 mg L^{-1} from the middle to the reactor outlet.

Figure 2. Aerial photograph of biological reactors (Guadalhorce WWTP). Source: Google Earth

The flocculated biomass, entering from reactors into the sedimentation tank, settles out of suspension forming the sludge and the clarified effluent. Some of the sludge in the clarifiers is returned to the reactor in order to maintain the microbial population at 2800 mg_{MLVSS} L^{-1}. The sludge recirculation ratio is adjusted hourly depending on the plant outflow rate. This plant was designed to operate at a food to micro-organism ratio (F/M) of 0.3 kg_{BOD} kg_{MLVSS}^{-1} d^{-1} associated with the sum of wastewater from the primary treatment and the returned AS. The excess sludge produced is submitted to anaerobic digestion. The target Solid Retention Time (SRT) is 4.5 days.

2.2. Respirometry measurements

Respirometry tests have been applied not only to samples of mixed liquor collected from inlet (FED) and outlet (UNFED) flow of reactor system but also to laboratory prepared samples. The synthetic samples were prepared mixing those from the influent wastewater to reactor or effluent wastewater and the returned activated sludge to simulate samples from inlet and outlet flow of reactor system, respectively. With the aim of studying endogenous respiration, sludge was submitted to aeration during 24 hours. The analyzed samples were collected in different days during the experimental time.

Respirometric tests were carried out with a BM-T Advance sludge respirometer supplied by the company Surcis. The setup basically consists of a 1 L capacity biological reactor, a dissolved oxygen meter, a peristaltic pump for recirculating samples and pH and temperature control. The system gives a set of measurements and calculations for decisive parameters approached to manage, design and research the biological process of wastewater treatment. This technology allows operating at three different modes: static, cyclic and dynamic. It should be highlight that for all measurements the temperature was maintained at 20.0 ± 0.1 ºC and the pH value within the range 7-8. The duration of experimental tests ranged from 0.5 to 1 hour. In this work, static mode was used with the aim of evaluating the biomass specific oxygen consumption rate while the dynamic respiration rate tests were carried out to determine the organic matter degradation rate and the amount of degraded organic matter.

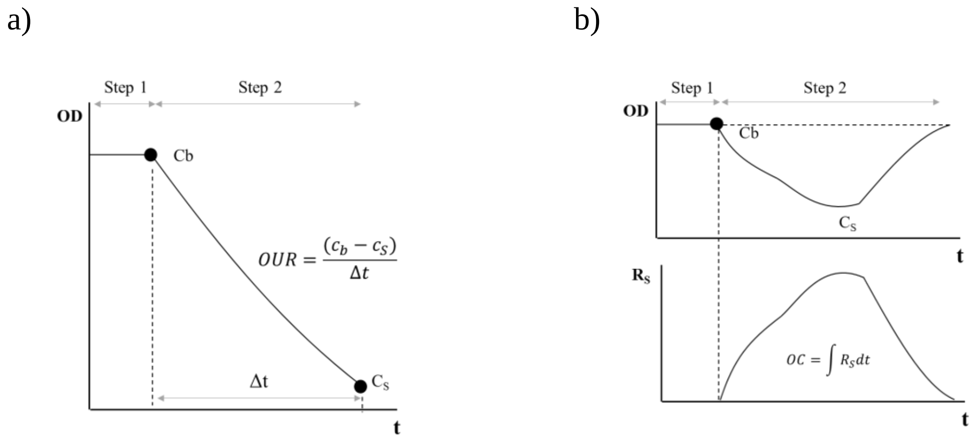

Figure 3. Respirometry test at: a) static mode and b) dynamic mode

Static respirometric tests were conducted to determine the Oxygen Uptake Rate (OUR). As shown in Fig. 3.a, the OUR value is obtained from a two-step procedure. In step 1, the dissolved oxygen (OD) is adjusted to the reference value C_b. During this step, the recirculation rate is high enough for considering the system as a complete mix reactor. In step 2, the aeration and recirculation is stopped and, consequently, the OD decreases to the final value C_s. The OUR value is directly obtained from the slope of this curve.

From the value of OUR, the Specific Oxygen Uptake Rate (SOUR) can be obtained as:

$$\text{SOUR}= \frac{OUR}{MLVSS}$$

Eq. 1

where MLVSS is the concentration of mixed liquor volatile suspended solids.

During dynamic test, it should be noted that the peristaltic pump and the aeration system do not stop until the test has finished, *i.e*, when all the biodegradable material has been completely consumed. Operating at dynamic mode, the Consumed Oxygen (CO) can be obtained in a two-step procedure as shown in Fig. 3.b. In step 1, it is adjusted the dissolved oxygen of reference (C_b). In step 2, the sample is added causing the decrease of the dissolved oxygen as a consequence of the biological oxidation. From the respirometric curve (R_s vs t), the values of dynamic respiration rate (R_{Smax} and $R_{Saverage}$) can be obtained. The consumed oxygen (CO) is obtained as the integral of Rs with respect to time.

The total Oxygen Uptake Rate during the endogenous phase (OUR_{end}) could be divided into heterotrophic ($OUR_{end.H}$) and autotrophic ($OUR_{end.A}$) biomass contributions. With the aim of obtaining the value of $OUR_{end.H}$, it is used the nitrification inhibitor: allylthiourea (ATU, 3-4 mg/g de MLVSS). Thus, for processes without nitrification, the total OUR_{end} is equal to $OUR_{end.H}$ and the OUR associated with the oxidation of organic substrate could be obtained as:

$$OUR_s = OUR_{FED} - OUR_{end.H} \qquad \textbf{Eq. 2}$$

where OUR_{FED} is the total oxygen uptake rate measured from the inflow of the biological reactor.

The chemical oxygen demand (COD) was determined using the spectrophotometer Dr. Lange DR5000 according to the standard method ISO 17025. The mixed liquor suspended solids (MLSS), the mixed liquor volatile suspended solids (MLVSS), the sludge volume index (SVI) and the biochemical oxygen demand (BOD) were determined according to standard methods for the Examination of Water and Wastewater [22].

2.3. Determination of biokinetic parameters of the heterotrophic biomass.

With the aim of evaluating microorganism activity in the activated sludge, the yield coefficient of heterotrophic biomass referred to chemical oxygen demand is studied ($Y_{H.COD}$) under dynamic operation of respirometer. The samples used were the sludge from the outflow of biological reactor under endogenous conditions (aeration during 24 hours) and, also, under normal conditions to compare. Two different experimental procedures varying in the substrate: sodium acetate or wastewater from the influent to the biological reactor, were applied. All these tests were carried out about the same pH, temperature conditions and MLVSS concentration as the actual process.

For the determination of $Y_{H.COD}$ using sodium acetate: A volume of 40-50 mL of a sodium acetate solution (400 mg $L^{-1} \approx$ 275 mg COD/L) was added in 1 L of endogenous sludge. From respirometry tests, the consumed oxygen (CO) was obtained.

For determination of $Y_{H.COD}$ using as substrate samples from wastewater, since this parameter is only associated with heterotrophic biomass, it was required to add allylthiourea (ATU) to inhibit the autotrophic biomass. The procedure basically consisted of: collection and filtration (<0.45μm) of samples from the influent to the biological reactor and from the final effluent; determination of *CODs* of samples from the influent to the biological reactor and from the final effluent, S_{in} and S_{ef}, respectively; calculation of *CODs* removed as $S_e = S_{in} - S_{ef}$; and, respirometry tests applied to filtered samples from the influent to the biological reactor to determine the consumed oxygen (*CO*).

The $Y_{H.COD}$ was determined as [23,24]:

$$Y_{H.COD} = 1 - \frac{CO}{COD_{s\_removed}} \qquad \textbf{Eq. 3}$$

where $COD_{s\_removed}$ was COD of sodium acetate solution or S_e for the methods carried out with sodium acetate and wastewater respectively.

This coefficient could be expressed as mass of *VSS* in relation with the consumed substrates expressed as *COD* mg $_{MLVSS}$ mg $_{COD}^{-1}$:

$$Y_{H.VSS} = \frac{Y_{H.COD}}{f_{cv}} \qquad \textbf{Eq. 4}$$

where the value of f_{cv}, the COD to VSS ratio, generally accepted is 1.42 $mg_{CODbact.}$ mg_{MLVSS}^{-1} [25].

Other parameter associated with $Y_{H.COD}$ is the observed heterotrophic cellular yield coefficient, Y_{obs} (mg $_{MLVSS}$/mg $_{CODs}$), which is used to estimate the sludge net production, obtained as [26]:

$$Y_{obs} = \frac{Y_{H.VSS}}{(1 + k_d \cdot SRT)} \qquad \textbf{Eq. 5}$$

where k_d (d^{-1}) is the biomass fraction oxidized per day during endogenous respiration with a typical value $k_d \approx 0.06$ d^{-1} and SRT (d) is the solid retention time.

The heterotrophic cellular decay rate in endogenous respiration, b_H (d^{-1}) can be obtained as [27]:

$$b_H = 0.24 \cdot 1.03^{(T-20)} \qquad \textbf{Eq. 6}$$

where T (ºC) is the temperature in the plant.

or by Ekama Method [28]:

$$b_H = \frac{k_d}{(1 - Y_{H.VSS}(1 - f_p))} \qquad \textbf{Eq. 7}$$

where f_p: Particulate biomass fraction ≈ 0.08.

The active heterotrophic biomass (X_H, mg L^{-1}) was determined from the value of $OUR_{end,H}$ (mg L^{-1} h^{-1}) as [29]:

$$X_H = \frac{24\left(\frac{h}{d}\right) \cdot OUR_{end.H}}{(f_{cv} \cdot b_H)} \qquad \textbf{Eq. 8}$$

The values of COD utilization rate (U, mg L^{-1} h^{-1}) and the specific COD utilization rate (q, mg_{COD} mg_{MLVSS}^{-1} d^{-1}) are obtained directly from the respirometer tests. Since these values are obtained under maximum oxygenation conditions, it must be corrected applying the half-saturation constant, K_{OD}, to obtain U and q under the influence of the concentration of dissolved oxygen U_{OD} and q_{DO} respectively [29]:

$$U_{DO} = U \cdot \left(\frac{DO}{(K_{DO} + DO)}\right) \qquad \textbf{Eq. 9}$$

$$q_{DO} = q \cdot \left(\frac{DO}{(K_{DO} + DO)}\right) \qquad \textbf{Eq. 10}$$

where U is the maximum value of the CODs utilization rate (mg_{CODs} L^{-1} h^{-1}) and q is the maximum value of specific CODs utilization rate (mg_{COD} mg_{MLVSS}^{-1} d^{-1}), both obtained from the respirogram.

The value of the half-saturation constant for dissolved oxygen is $K_{DO} \approx 0.1$. Although this value can be omitted, ($K_{DO} = 0$), when the medium value of dissolved oxygen (DO, mg L^{-1}) is more than 2 mg L^{-1}

From the respirograms, it is also possible to estimate the half-saturation constant K_S (mg L^{-1}) following the Michael-Menten equation and the real COD utilization rate in the biological process (U_{act}, mg_{COD} L^{-1} d^{-1}) as:

$$U_{act} = \frac{COD_e}{HRT} \qquad \textbf{Eq. 11}$$

where COD_e is the real COD removed in the biological reactor (mg L^{-1}) and HRT is the hydraulic retention time (d).

The growing rate of the heterotrophic biomass (μ_{H}, d^{-1}) is associated with the active heterotrophic biomass concentration (X_H) [30]:

$$\mu_H = Y_{H.VSS} \cdot \left(\frac{U_{act}}{X_H}\right) - b_H \qquad \textbf{Eq. 12}$$

$$\mu_{Hmax} = \mu_H \left(\frac{OD + K_{OD}}{OD}\right) * \left(\frac{S + K_S}{S}\right) \qquad \textbf{Eq. 13}$$

where:

S: COD of dissolved substrate in the biological reactor, mg L^{-1}.

3. Results and discussion

3.1. Evaluation of Sludge Activated

Results from the respirometry tests applied to samples of mixed liquor collected from inlet (FED) and outlet (UNFED) flow of the biological reactor (fresh samples) together with values obtained from laboratory prepared samples (synthetic samples) are shown in Table 1. These experiments were carried out during several months.

Table 1. Results of Respirometry tests applied to fresh and synthetic samples.

	Date	Sample	MLSS	MLVSS	Volatile	OUR	SOUR	F/M	FC	HRT	SRT	R
			g L^{-1}	g L^{-1}	%	mg L^{-1} h^{-1}	mg g^{-1}h^{-1}	g$_{BOD}$ g$_{MLVSS}$$^{-1}$ d^{-1}		h	d	%
Fresh samples	10/14/16	FED	2.4	2.1	88.2	32.3	15.4	0.7	1.2	3.9		70
		UNFED	2.5	2.1	83.9	26.6	12.7					
	10/18/16	FED	3.4	3.1	90.1	43.7	14.1	0.8	0.9	3.6	2.6	135
		UNFED	3.3	2.9	87.8	47.0	13.3					
	10/25/16	FED	3.8	3.4	89.5	36.3	10.7	0.6	1.1	3.6	5.1	135
		UNFED	3.9	3.4	87.0	32.1	9.5					
	12/27/16	FED	4.2	3.3	78.0	46.0	11.9	0.7	1.0	3	4.7	135
		UNFED	4.7	4.1	86.0	44.1	10.9					
	04/12/17	FED	5.8	4.8	83.0	39.0	8.5	0.5	1.0	3.7	2.3	130
		UNFED	4.6	3.9	84.0	37.7	9.7					
	05/15/17	FED	3.4	3	88.4	34.0	11.2	0.7	1.5	3.3	2.5	90
		UNFED	3.1	2.7	88.4	22.0	8.0					
Synthetic samples	10/25/16	FED	4.0	3.6	90.4	38.0	10.6	0.6	1.0	3.6	5.1	135
		UNFED	4.2	3.8	89.6	39.0	10.3					
	11/04/16	FED	4.0	3.6	89.6	32.2	8.9	0.6	1.0	3.8	3.9	135
		UNFED	4.1	3.6	87.8	31.7	8.8					
	11/29/16	FED	2.5	2.2	89.4	39.7	18.0	0.5	1.4	4.0	3.7	90
		UNFED	2.4	2.1	88.9	28.0	13.1					

As can be observed, there are no important differences between the values of OUR and SOUR obtained for fresh samples from sampling point in the biological reactor of the plant and synthetic samples. Results of the OUR and SOUR from samples in the inlet (FED) and outlet (UNFED) flow from the biological reactor are dependent on the recirculation rates (R). For the higher values of R, 130-135%, the OUR and SOUR values were similar for FED and UNFED samples, i.e, secondary treatment operates similarly to a continuously stirred tank reactor (CSTR). However, for lower values of R, 70-90%, the values of OUR for FED and UNFED samples were different, i.e., biological reactors operate similarly to a plug flow reactor (PFR).

With the aim of evaluating the biological process, the factor charge (FC) as the ratio of OUR from inlet (FED) to outlet (UNFED) samples from the biological reactor has been obtained. Thus, comparing experimental results of FC (Table 1) with values shown in Table 2a, it could be concluded that the diagnostic is: “Low Efficiency or low loading”.

However, to understand these results, it should be noted that all the experimental F/M values (

Table 1) were above 0.5 $kg_{BOD.}\ kg_{MLVSS}^{-1} d^{-1}$ which indicates that the organic load was not low, excluding the assessment of low loading.

On the other hand, for the design operating conditions (F/M=0.3 kgBOD $kgMLVSS^{-1}$ d^{-1} and SRT=4.5 days.) the results should present values of SOUR for UNFED samples from 7 to 10 mgO_2 g^{-1} h^{-1}. However, most of the samples (

Table 1) are above these reference values, leading to the assessment of “Overloading”.

Table 2. Values of reference. [1]for UNFED SOUR below reference values [31].

a)

FC	**Assessment**
<1	Toxic or inhibitory symptoms
1<FC<2	Low efficiency[1] or low loading
2<FC<5	Aceptable loading
FC>5	Possible overloading

b)

Process Type $kg_{BOD}\cdot kg_{MLVSS}^{-1}\ d^{-1}$	**SRT** d	**UNFED SOUR Reference value** $mg_{O2}\ g^{-1}\ h^{-1}$	**UNFED SOUR Experimental value**	**Assessment**
High loading F/M > 0.4	2-4	10-15	>> reference values	Overloading
Medium loading 0.2 < F/M < 0.4	4-10	7-10	≈ reference values	Good performance

Low loading 0.07 < F/M < 0.2	10-30	3-7	< reference values	Low loading
Extended aeration F/M < 0.07	10-30	3-7	<< reference values	Very low loading or toxicity symptoms

The evaluation of sludge under endogenous respiration was carried out through OUR and SOUR tests applied to the samples from the biological reactor outflow. The results associated with samples untreated and treated with ATU are shown in Table 3.

Table 3. Results of respirometry tests applied to endogenous samples.

Date	**Sample**	**MLSS**	**MLVSS**	**Volatile**	**OUR$_{end}$**		**OUR$_{end.A}$**
		g L^{-1}	**g L^{-1}**	**%**	**mg L^{-1} h^{-1}**		**mg L^{-1} h^{-1}**
					without ATU	**with ATU**	
04/18/17	UNFED	4.3	3.8	89	22.1	15.3	6.8
05/09/17	UNFED	4.2	3.7	89	17.0	3.6	13.4
06/06/17	UNFED	2.8	2.5	88	15.2	8.5	6.7

As it was aforementioned, the total oxygen rate during the endogenous phase (OUR$_{end}$) should be equal to the OUR associated with the heterotrophic biomass contributions for processes without nitrification (OUR$_{end.H}$). However, the comparison of OUR values obtained with and without nitrification inhibitor (ATU) put forward that the process was taking place with nitrification, although this was not included in the design of the plant.

The value of OUR associated with autotrophic biomass (OUR$_{end.A}$) is obtained as the difference of total OUR$_{end}$ and OUR$_{end.H}$. Comparing experimental results of OUR$_{end}$ with reference values associated with the concentration of volatile suspended soils in sludge [31], the results obtained when samples are treated with ATU are within the normal range. However, for samples not treated with ATU, the values of OUR$_{end}$ are above reference levels. These results are also an indicative of the presence of nitrifying activity of the autotrophic bacteria.

Aiming at evaluating sedimentation of activated sludge, the sludge volume index (SVI) was measured during almost 10 months. Results of SVI versus F/M are shown in Fig. 4.

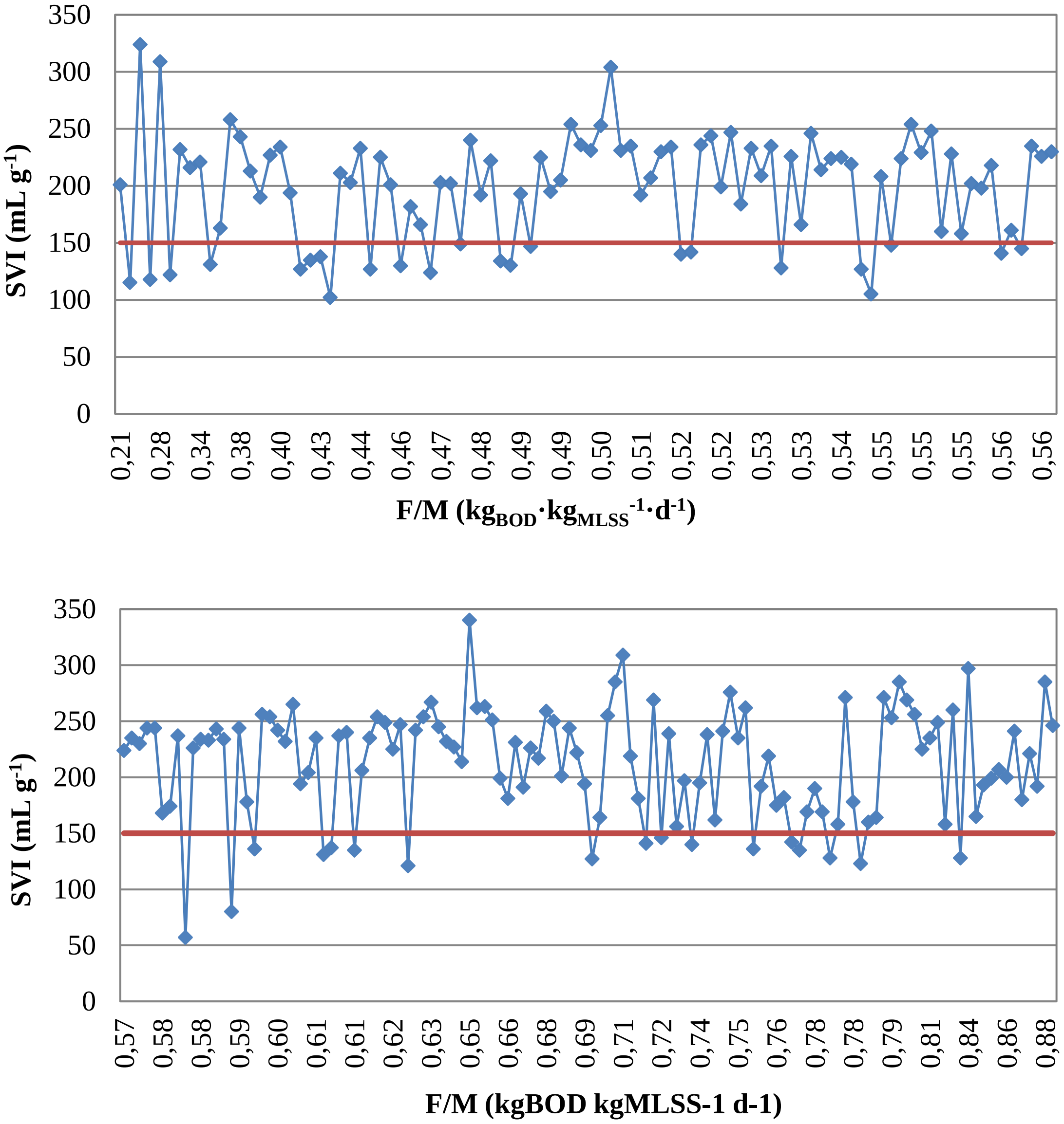

Figure 4. Values of SVI versus F/M.

Although typical values of SVI in municipal wastewaters ranges from 50 to 150 mL g^{-1} [32], it is difficult to estimate the maximum value allowed of SVI since the clarifier efficiency depends on other parameters, such as: clarifier dimensions, recirculation rate, effluent composition and MLSS. The average values of SVI, F/M and MLSS obtained along the experimental time were 208 mL g^{-1}, 0.6 kg$_{BOD}$ kg$_{MLVSS}$$^{-1}$d^{-1} and 3545 mg L^{-1}, respectively. As can be observed in Fig. 4, most of the SVI values (≈80 %) are above 150 mg L^{-1}. Values of SVI below 150 mg L^{-1} and with an acceptable F/M value (<0.6 kg$_{BOD}$ kg$_{MLSS}$$^{-1}$d^{-1}) are only 12% of total samples. From experimental results, important differences are found among experimental values of F/M and MLVSS and target values of this wastewater plant (0.3 kg$_{BOD}$ kg$_{MLVSS}$$^{-1}$ d^{-1} and 2800 mg L^{-1}, respectively). However, it should be noted that SVI values rarely reached 400 mL g^{-1}, value from which the number of crawling ciliates is reduced drastically [33]. These microorganisms together with sessile ciliates play an important role on particle aggregation, being one of the most important population of biological communities in AS. The primary role of ciliates in the biological treatment is the clarification of the effluent. It has been experimentally proved that the absence of ciliates entails effluent with higher BOD and turbidity as a consequence of the presence of many dispersed bacteria [34]. Therefore, the control of the experimental conditions is essential to increase the efficiency of particle aggregation. Moreover, as ciliates feed on pathogenic and fecal bacteria, the presence of Escherichia coli is reduced to 5 % when ciliates are present [35]. Regarding other parameters with influence on the ciliates presence, for values of F/M above 0.6 $kg_{BOD}\ kg_{MLSS}^{-1}d^{-1}$ most species of crawling ciliates disappears [36].

The excessive growth of filamentous bacteria, known as filamentous bulking sludge, is other problem frequently found in AS process that directly affects to the operational performance. According to Guo et al. [37] the value of SVI could be used to diagnose this problem concluding that SVI values higher than 250 mL mg^{-1} entails serious filamentous bulking. The main approach to avoid this problem is the control of operational conditions, such as: solid retention time, food to microorganism ratio, F/M and oxygen concentration. According to the above-mentioned, the detected plant operation under overload conditions could cause the proliferation of filamentous bacteria which implies problems in secondary decantation.

In conclusion, the overload conditions are not only impacting on the efficiency of biological treatment but also on the operation of the secondary clarifier.

3.2. Determination of stoichiometric coefficients and biokinetic parameters of the heterotrophic biomass.

With the aim of evaluating the global performance of the plant, the main properties of samples collected in the inflow of the biological reactor and those from the plant outflow are shown in Table 4.

Table 4. Wastewater characteristics at the inflow and outflow of the WWTP.

Parameter	Inflow	Outflow
Dissolved oxygen (mg L^{-1})	Average: 0.92	Average: 1.65
COD (mg L^{-1})	444	74
BOD_5 (mg L^{-1})	230	20
Ammonium (mgN-NH_4 L^{-1})	49	44

Nitrate (mgN-NO_3 L^{-1})	0.3	0.7
Nitrite (mgN-NO_2 L^{-1})	0	0.2
Total nitrogen (mgN L^{-1})	72	57
Total phosphorus (mgP L^{-1})	10	3

The values of COD and BOD for samples collected in the inflow of the biological reactor are within the usual range for urban wastewater which are: 250-800 mg L^{-1} and 100-300 mg L^{-1} for COD and BOD_5, respectively [38].

Thus, the COD and BOD removal efficiency of the biological treatment was good reducing the initial concentration to 17 and 9 % of the initial concentration of COD and BOD, respectively.

The main results obtained from respirometry tests following the procedure described in 2.2 together with some operational parameters of the plant are presented in Table 5. The samples analyzed were mixed with wastewater from the influent to the AS process and with one standard of sodium acetate under different conditions with and without ATU and fresh and under endogenous respiration

Table 5. Summary of main operational parameters of the plant and results obtained from respirometry test.

MLSS (g L^{-1})	2.78
MLVSS (g L^{-1})	2.46
Volatile (%)	88
WWTP T(ºC)	25
Respirometer T(ºC)	20.5
F/M (kg$_{BOD}$·kg$_{MLSS}^{-1}$ d^{-1})	0.80
HRT (h)	3.3
SRT (d)	1.8
U$_{act}$ (mg$_{COD}$ L^{-1} h^{-1})	112

	Sodium Acetate				Wastewater
	Fresh Sludge		Endogenous Sludge		
ATU	No	Yes	No	Yes	Yes
OUR (mg$_{O_2}$ L^{-1} h^{-1})	22.0	23.1	15.2	8.5	
SOUR (mg$_{O_2}$ g^{-1} h^{-1})	8.9	9.4	6.2	3.5	
OUR$_S$ (mg$_{O_2}$ L^{-1} h^{-1})		14.6			
OUR$_{end.A}$ (mg$_{O_2}$ L^{-1} h^{-1})			6.7		
R$_{Smax}$ (mg$_{O_2}$ L^{-1} h^{-1})	32.7	31.7		24.3	8.7
CO (mg$_{O_2}$ L^{-1})	99.4	87.6		67.6	18.8
COD (mg$_{O_2}$ L^{-1})	301.0	265.6		204.8	57.0
$Y_{H.COD}$ (mg$_{CODbact}$ mg$_{CODs}^{-1}$)	0.67	0.71		0.78	0.86
$Y_{H.VSS}$ (mg$_{MLVSS}$ mg$_{CODs}^{-1}$)	0.47	0.50		0.55	0.61
Y_{obs} (mg$_{MLVSS}$ mg$_{CODs}^{-1}$)	0.43	0.45		0.50	0.55
U$_{max}$ (mg$_{COD}$ L^{-1} d^{-1})	65.2	62.5		42.3	19.8
q$_{max}$ (mg$_{COD}$ mg$_{MLVSS}^{-1}$ d^{-1})	0.64	0.61		0.41	0.19
U$_{OD}$ (mg $_{COD}$/L·d)				35.64	16.7
q$_{OD}$ (mg$_{COD}$ mg$_{MLVSS}^{-1}$ d^{-1})				0.34	0.17

k_d (d^{-1})	0.06
b_H (d^{-1})	0.21-0.24
X_H (mg$_{COD}$ L^{-1})	695
K_S (mg$_{COD}$ L^{-1})	5.97
μ_H (d^{-1})	1.92-2.51
μ_{Hmax} (d^{-1})	2.28-2.98

As can been observed, SOUR values for fresh sludge analyzed in presence of sodium acetate with and without ATU were about 9 mgO$_2$ g^{-1} h^{-1}, higher than references values (4 – 7 mg$_{O_2}$ L^{-1} h^{-1}). Also, the experimental value of OUR$_{end}$, 15.2 mg$_{O_2}$ L^{-1} h^{-1}, is higher than the reference values abovementioned which could be related with the system overload. The value of OUR$_s$, 14.6 mg$_{O_2}$ L^{-1} h^{-1}, obtained as the difference between OUR of the fresh sludge and OUR_{end} (both with ATU) highlighted the great difference between the two samples. The endogenous respiration rate of the autotrophic biomass has been obtained by the difference between the total OUR_{end} and the $OUR_{end.H}$. Thus, the value obtained of $OUR_{end.A}$, 6.7 mg_{O2} L^{-1} h^{-1}, reflects the important contribution of the nitrifiers to endogenous respiration rate (≈ 44%). Regarding maximum dynamic respiration rate, R_{Smax}, the values obtained were 24.3 and 8.7 mg_{O2} L^{-1} h^{-1} for endogenous sludge with ATU in presence of sodium acetate and wastewater, respectively; i.e., the reduction in the biomass activity in wastewater was of 64% compared with the reference value obtained with sodium acetate. Thus, the decrease is associated with the wastewater instead of with the biomass.

Regarding biokinetic parameters, there are no important differences for values obtained for fresh sludge samples in presence of sodium acetate with and without adding ATU. These results are different from those obtained for endogenous sludge. At the same time, parameters obtained for endogenous sludge with and without ATU present important divergences. The $Y_{H.COD}$ for sludge samples in presence of sodium acetate, a completely biodegradable compound, and in presence of wastewater are also collected in Table 5. The value of $Y_{H.COD}$ for sludge samples in presence of sodium acetate under endogenous respiration and adding ATU was 0.78 $mg_{CODbact}$ mg_{CODs}^{-1} within the reference range (0.75-0.79) [39]. The value of this parameter for wastewater adding ATU was slightly higher (0.86 $mg_{CODbact}$ mg_{CODs}^{-1}). These results indicate the sludge was not found under toxic effects. Expressing the aforementioned coefficient as mass of VSS in relation to the consumed subtracts as COD, $Y_{H.VSS}$, the values obtained for the aforementioned samples were 0.55 and 0.61 mg_{MLVSS} mg_{CODs}^{-1}, respectively. These values are in agreement with experimental results obtained by other authors [40]. Other parameter associated with $Y_{H,COD}$ is the observed heterotrophic cellular yield coefficient, Y_{obs}, whose values were 0.50 and 0.55 mg_{MLVSS} mg_{CODs}^{-1} for samples of endogenous sludge adding ATU in presence of sodium acetate and wastewater, respectively, close to usual reference range (0.3-0.5 mg_{MLVSS} mg_{CODs}^{-1}) [41]. The value of active heterotrophic biomass, X_H, was 695 mg_{COD} L^{-1}, corresponding to 28% of MLVSS, which is slightly higher than referenced values (14-25%) [40].

The specific COD utilization rate (q_{max}) obtained for sludge samples in presence of sodium acetate and wastewater was 0.41 and 0.19 mg_{COD} mg_{MLVSS}^{-1} d^{-1}, respectively. The observed difference suggested that the reduction in the activity and in the rate of COD degradation is associated with the influence of the wastewater instead of the biomass from activated sludge. As was explained in 2.3, since the values of q and U are obtained under maximum oxygenation conditions, these should be corrected using the half-saturation constants, K_{OD}, to obtain U and q under the influence of the concentration of dissolved oxygen U_{OD} and $q_{OD,}$ respectively. The values of this parameters were $U_{OD} = 35.64$ mg COD L^{-1} h^{-1} and $q_{OD}= 0.35$ mg_{COD} mg_{MLVSS}^{-1} d^{-1} for samples with sodium acetate and $U_{OD}= 16.7$ mg COD L^{-1} h^{-1} and $q_{OD} = 0.16$ mg_{COD} mg_{MLVSS}^{-1} d^{-1} for samples with wastewater.

The result obtained here from respirometry tests for half-saturation constant, K_S, 5.97 mg L^{-1} is within range of the typical values for urban wastewater 5 to 40 mg mL^{-1} [42] and the maxima growing rate of the heterotrophic biomass ($\mu_{Hmax,}$ d^{-1}) = 2.28-2.98 is also quite similar to the reported values by Koch et al. [43] or Guisasola et al. [44].

4. Conclusions

The use of respirometry tests in a real WWTP has provided important information to diagnose the performance of activated sludge process. Experimental results suggested that the plant was operating at low efficiency and at overload. The overload conditions have been found to impact not only on the efficiency of biological treatment but also on secondary clarifier operation. Regarding the oxygen consumed, it has been detected a significant increase related to the nitrification process despite not being considered in the initial design of the plant. The reduction in the biomass activity in wastewater was of 64% compared with the reference value obtained from sodium acetate. Thus, the decrease of the activity is associated with the wastewater instead of with the biomass. Although the WWTP operation conditions are not the ideal, it should be noted that the plant complies with the legal limits of emission and with the required yields in the discharge permission and in the current Spain regulations.

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