ESTIMATION OF AVAILABLE BIODEGRADABLE SUBSTRATE (ABS): ALTERNATIVE METHOD

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The five days Biological Oxygen Demand (BOD_5), when related to its COD concentration, is usually used to express the wastewater biodegradability. However, it does not allow to estimate the time required for its degradation and it does not distinguish between rapidly or slowly biodegradable substrates. The reference time for biodegradability assessment is also related to the retention time (hours) of the substrates within the biological reactor, which is very different from the BOD test duration (five days). Innovative methods were developed to improve the determination of the biodegradable and/or readily biodegradable wastewater fraction by comparison of the test response with known substrates.

The present study provides an alternative and complementary method to the traditional BOD. The proposed method is based on the evaluation of the biomass oxygen consumption by degradation of the available biological substrate (ABS) into a wastewater, through respirometric tests simulating the biological reactor of the treatment plant at laboratory scale. This method also allows to obtain the actual oxygen consumption for the biodegradation of organic substrate and to evaluate the toxic effect of aqueous waste to biomass.

The results obtained show an inverse relationship between the COD fraction that is biodegraded by the hydraulic retention time (HRT) that characterises the oxidative reactor and the organic load fed to biomass. Starting from oxygen uptake/COD ratio of 0.40, obtained with a low organic substrate concentration, 0.05 with high concentration is achieved.

Keywords: Biological Oxygen Demand (BOD); biodegradable fraction; respirometric test; aqueous waste; management of wastewater treatment plants.

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1. Introduction

Aqueous waste can contain significant quantities of pollutants depending on the production process that generated them [1]. These substances can be treated with conventional or advanced biological processes [2,3], chemical treatments [4,5] or the combination of both [6,7].

Aqueous waste produced by industrial processing are:

(i) treated within the same facilities unit (with the subsequent discharged into water bodies or in sewer), if suitable treatments for aqueous waste are available

(ii) transported by tank truck in traditional wastewater treatment plants (WWTPs) or in a specific pre-treatment station for aqueous waste.

Whatever the destination, the characterization of pollutants and a more clearly definition of the specific treatments, are essential to ensure an optimal and careful management of these.

Furthermore, since WWTPs that treat the aqueous waste usually provide for biological processes, it is important that during waste characterization the biological treatability is firstly verified. Therefore, the evaluation of the long-term biodegradable organic content (5-20 days) and the effectively biodegradable fraction under the operating conditions of the treatment plant is very important.

The BOD is the parameter that, compared to its respective COD, is used to assess the biodegradability of a wastewater as it represents the biodegradable organic matter referred to total organic substance. In the industrial field, BOD determination becomes more uncertain and often it is not interpretable as the analogous measure carried out on urban wastewater. The main factors affecting the ineffectiveness of measurement are: the presence of compounds, usually absent in urban wastewater, which can alter the metabolic chain, and the toxic substances, that inhibit bacterial activity. Therefore, they have a negative role in quantifying the actually contained biodegradable substance. Although no toxicity effects are occurred, the presence of organic matter that is refractory to biodegradation from bacterial strains may amend the COD/BOD ratio.

Conventional methods for the BOD test do not allow the assessment of actually biodegradable substrate fraction by WWTP, because sewage hydraulic retention time HRT (few hours) is different to the length of BOD test (equal to 5 days). This standardized method shows some limitations:

- a significant variability in the results (>20%), mainly due to microbial population was observed;
- the long period of analysis (5 days) appears inappropriate for the online monitoring of processes found in WWTP;
- the measurement range of the organic load is limited by the amount of dissolved oxygen (DO).

The limitations of manometric method concerned the long period of analysis, the measurement variability due to inoculum quality and the indirect measurement of pressure may affect the results [8,9]. Moreover, the biomass "operational capacity" on biological process is different compared to operational conditions of manometric test: residence time is completely different to HRT of bioreactor. BOD_5 of an aqueous waste containing a high amount of biodegradable organic matter that can be inhibited by different factors as toxicity, lack of nutrients, absence of adapted microorganisms which causes an erroneous low value of this measurement [10]. Finally, the conventional BOD test is not suitable for online monitoring of WWTP, due to long duration of measurement [11].

During the years, researchers have developed technological innovations that aim to expedite the BOD measurement and making it more practicable for management of the biological stage in WWTP [12]. The alternative measures proposed include biosensor tests [13,14], biosensor with redox-mediator [15], fluorescence technique [16] and microbial fuel cells [17]. In addition, tests based on respirometry allow both the measurement and interpretation of oxygen consumption mode to remove the biodegradable substrates by biological treatment [18,19]. Measurement and interpretation of biological oxygen consumption rate under experimental conditions are the base of aerobic respirometry [20,21]. It provides important information through the determination of kinetic and stoichiometric parameters for optimized design (volume), operating conditions (oxygen supply) and upgrading of the biological stage in a WWTP [22,23]. Moreover, those techniques are used to determine BOD [24], COD fraction [25], toxicity and inhibitory effects of some residues in wastewater [26].

The aim of this work is the proposal of alternative method for the evaluation of biodegradable fraction in a substrate available for the biomass of the biological process (available biodegradable substrate - ABS), in relation to the actual operating conditions of WWTP that receive such substrate.

The proposed method also allows: (i) identifying the possible toxic or inhibitory effects of substrate on bacterial biomass of WWTP; (ii) determining of real oxygen demand required for substrate biodegradation within the WWTP biological reactor.

2. Material and methods

Respirometric technique is widely used as an alternative method for the evaluation of biodegradable fraction in a wastewater. Respirometry allows to measure rapidly and slowly biodegradable fraction into organic substrate, through laboratory techniques that simulate the activated sludge treatment. This method is both a viable alternative and an integration to traditional BOD test, and it also allows to evaluation of the real biodegradable fraction available to biological treatment.

The alternative tests were carried out with non-synthetic aqueous waste having significant biodegradability, according to standard BOD measures. The results refer to aqueous waste treated by an AWTP plant (Aqueous Waste Treatment Plant), located in northern Italy and are shown below. The aqueous waste line includes several chemical-physical pre-treatment steps followed by a pure oxygen thermophilic biological membrane reactor [27]. Effluent characteristics are shown in Table 1.

Table 1

Sample average concentration

Average parameters	**pH**	**COD**	**BOD_5**	**$N\text{-}NH_4^+$**	**$N\text{-}NO_3^-$**	**$N\text{-}NO_2^-$**	**TP**
		[mg L^{-1}]	[mg L^{-1}]	[mg L^{-1}]	[mg L^{-1}]	[mg L^{-1}]	[mg L^{-1}]
Sample effluent	7.5	1,650	300	25	10	1	10

The WWTP that receives the aqueous waste pre-treated in the AWTP described above, is in northern Italy; it consists of a classic active sludge process with pre-denitrification and primary sedimentation. This plant, with a capacity of 120,000 population equivalent, receives both urban wastewater and aqueous waste (pre-treated) from a sewer. The average input COD concentration is approximately of 300 mg L^{-1}. Mesophilic biomass used for the test comes from this plant and, according to alternative method, the operational parameters of this WWTP were considered, especially HRT of biological treatment. Total suspended solids (TSS) of biomass is equal to 3 g L^{-1}, that 70% is volatile (VSS).

COD, nitrogen compounds, total phosphorous and suspended solids were measured according with Italian standard methods [28]. The traditional BOD test is based on direct measurement of DO in wastewater sample, diluted or undiluted, before and after five days incubation at 20 °C, according to International Standard method ISO 5815-1:2003 [29]. During this experimentation, manometric method is used for assessing BOD_5, based on the measurement of pressure decreased obtained from oxygen consumption by microorganisms oxidizing the biodegradable matter. The pressure changes are measured with pressure sensor and convert to BOD by the device on the measurement heads that closes the analysis bottles hermetically.

The alternative method for the evaluation of ABS (available biodegradable substrate) in a wastewater is based on respirometric test. The laboratory equipment is reported in Fig. 1; the DO measurement is performed by means a WTW Sentix 940-3 probe.

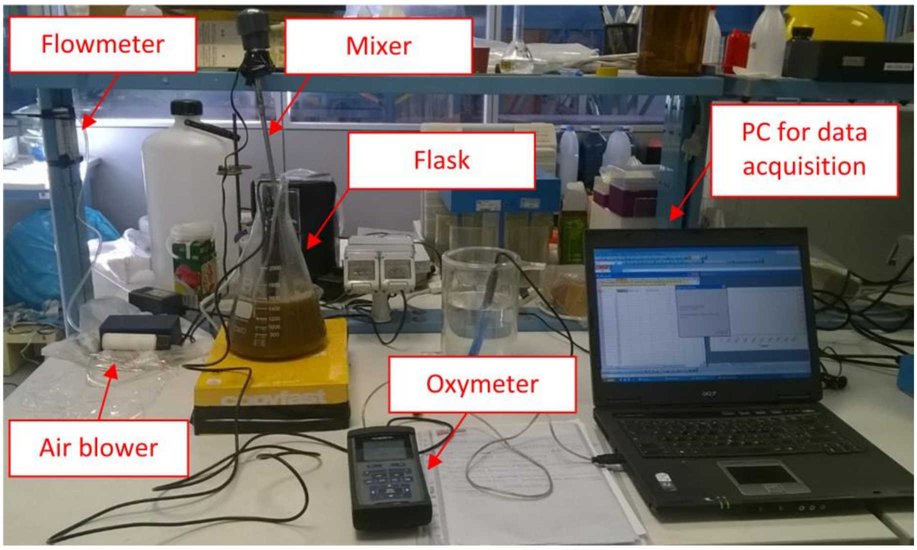

Fig. 1: Laboratory equipment used for the evaluation of ABS

The proposed methodology includes the following procedural steps:

- preliminary activity, where mixed liquor (collected from oxidation reactor) is maintained in continuous aeration for 24 hours to obtain endogenous breathing condition;
- introduction of biomass into flask, activation of constant air flow for oxygenation by means of flowmeter, until the concentration reaches about 8 mg_{O2} L^{-1};
- suspension of aeration and isolation of mixed liquor contained into flask from the outside air through a cotton swab that also minimizes the head space, for the evaluation of endogenous biomass respiration curve for simply vital functions (Fig. 2 – section 1);
- reactivation of oxygenation, by maintaining the same flux, and measure the reoxygenation curve, in which there is still the contribution of endogenous respiration (Figure 2 – section 2);
- continue the curve until the asymptote and, subsequently, dose the known volume of substrate to be tested and detection of characteristic sag curve (Figure 2 – section 3), that allows acquisition of necessary data for calculation of ABS; the trend of this section depends on the oxygen consumption of biomass (oxidation of substrate and endogenous respiration), by also the reoxygenation of the system;
- the test continues to the equilibrium conditions, in which oxygen concentration trend remains constant.

The length of the sag curve (section 3) is identified equal to HRT of the biological reactor, in which it is expected to treat the analyzed wastewater. This may determine effective concentration of biological substance made available to biomass in the real operating conditions. These characteristics are the main differences compared to traditional BOD measurement, which provides an independent data to the real operating conditions of biological reactor.

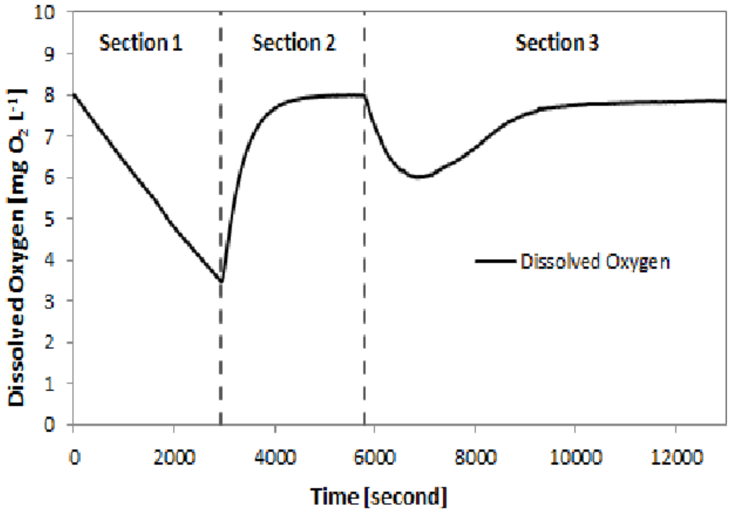

Fig. 2: DO concentration on respirometric test for the evaluation of ABS

These three curves are processed using a calculation sheet that determines the different kinetics of biomass respiration, shown below.

3. Results

The Fig. 3a, concerning the endogenous respiration (section 1), shows the real DO consumption and the straight line obtained by determining the coefficient K_1 [mg_{O2} L^{-1} min^{-1}], which represents the oxygen consumption rate only due to the endogenous respiration. This coefficient is calculated by minimizing the sum of the standard deviation between the real values and those modeled; the elaboration is performed in the section where the "reaction" processes according to a zero-order kinetic (non-limiting oxygen concentration), using Equation (1) where C represents the DO concentration over time [mg L^{-1}].

$$dC/dt = -K_1 \quad (1)$$

The section 2 of reoxygenation (Figure 3b) is elaborated through the DO concentration in the reoxygenation phase and the curve characterized by the coefficient K_2 [min^{-1}], which expresses the oxygen transfer velocity obtained by minimizing the sum of the standard deviation between the real values and those modeled (Eq. 2).

$$dC/dt = K_2 \cdot (C_e - C) \quad (2)$$

In Equation (2) C_e indicates the DO equilibrium concentration [mg L^{-1}].

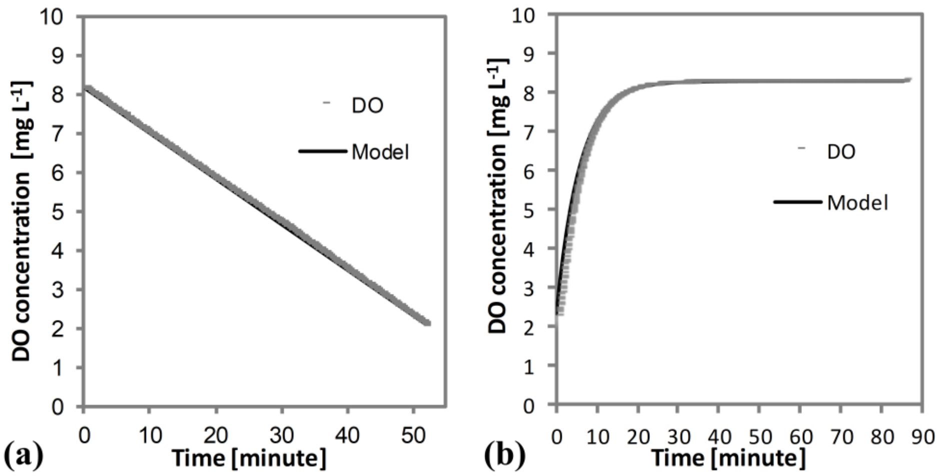

Fig. 3a,b: Development of first two test phases

The elaboration of sag curve (section 3), shown on Fig. 4, consists of DO concentration trend, the cumulated oxygen evolution in time (achieved as the difference between the oxygen consumed by biomass and the oxygen transferred through aeration) and the DO curve used instantly. Finally, the following equation (Eq. 3) is used to determine the ABS concentration [mg L^{-1}].

$$ABS = DO_{consumed} \cdot (\text{biomass Vol.} + \text{substrate Vol.})/\text{substrate Vol.} \qquad (3)$$

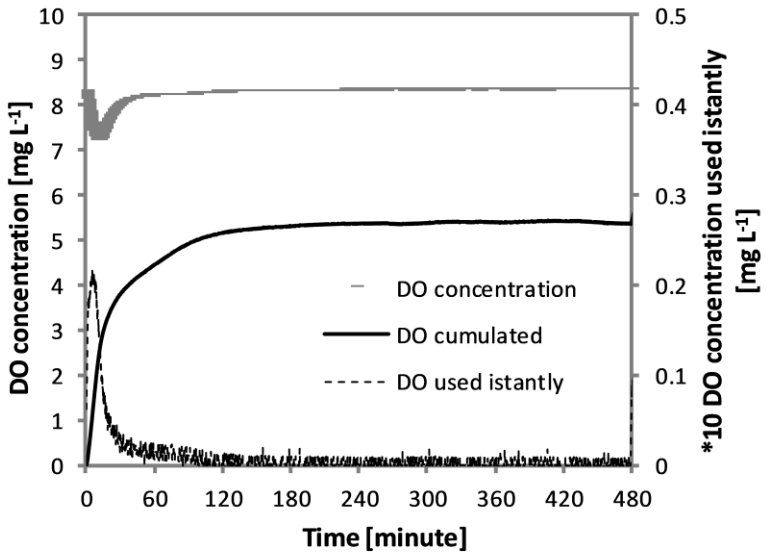

Fig. 4: Development of the sag curve (section 3 of respirometric test).

For the sag curve, the kinetic constant K_1 is recalculated at each step to obtain the asymptote of the final section curve in line with the asymptote of the previously measured reoxygenation curve.

The most significant operational parameters obtained during testing are:

- The *test duration* must be corrected; the third section must be longer than the time required to reach the equilibrium (represented by the straight line of DO concentration in the mixture). Early interruption may cause underestimation of the ABS value and the overall duration should not be upper than HRT of biological treatment which receives the substrate.
- The *air flow rate* (regulated with the flowmeter) has a significant influence on the sag curve, because high oxygenation rate involves a crush of the first portion of the same curve (section 3), making it difficult to estimate the oxygen consumption required for the substrate oxidation; moreover, the air flow shall remain constant during the test.
- The *amount of substrate* dosed within the test also involves sensitive variations, because excessive organic substrate intake results in a more pronounced sag curve, which could cause an excessively decrease of DO, thus making it difficult for the data processing; and, conversely a limited sample dosage results in a reduced oxygen consumption curve, which also leads in this case an unclear evaluation of kinetic parameters.
- The *temperature* inside the system should remain constant at 20 °C throughout the test, in order to avoid variations that could affect the DO concentration in the mixture.

During the experimentation, mixed liquor coming from the WWTP that receives the analyzed aqueous waste and sample of that wastewater were used, as previously described. Each analytical campaign consists of several respirometric tests carried out at different substrate dosage from 6 to 300 mL, repeated three times, keeping the mixed liquor volume unchanged and consequently the amount of biomass present in the blend, this last equal to 1,000 mL.

Table 2 includes, for every test, the K_1 and K_2 coefficients, C_e equilibrium concentration and ABS concentration obtained by alternative method, by maintaining operating conditions equal to the biological reactor (HRT of reactor). The results show a significant difference between the dosages adopted, in relation to the OD consumption used to degrade the added organic substance. In particular, increasing the substrate dosage, decreases the ABS value and therefore the oxygen load used to COD degradation. For the first three dosages the ABS fraction was between 30% and 40% of the total COD input, while the ABS concentration was reduced until 5% with the increasing COD load. The results obtained show that bacteria biomass biodegrades a higher COD fraction if fed with a low amount of substrate, compared to increased inoculum concentration. In the latter situation (test 7), there is a possible overload effect of the organic substance, which is not completely degraded during the test residence time and consequently within the biological reactor.

Table 2

Results of respirometric tests for the evaluation of the ABS fraction.

Tests	Substrate dosage	K_1	K_2	C_e	ABS	ABS/COD
	[mL]	[mg L^{-1} min^{-1}]	[min^{-1}]	[mg L^{-1}]	[mg L^{-1}]	[%]
1	6	0.117	0.183	8.85	**671**	**41**
2	12	0.117	0.171	8.98	**455**	**28**
3	25	0.087	0.134	8.86	**570**	**35**
4	50	0.083	0.161	8.85	**305**	**19**
5	100	0.102	0.118	8.88	**352**	**21**
6	200	0.097	0.134	8.94	**128**	**8**
7	300	0.102	0.167	9.09	**78**	**5**

The higher dosages, though increasing the oxygen demand by the treatment plant, causes a net decrease in the biodegradability of COD fed, showing a suffering or overloading situation of biomass. Actually, high aqueous waste dosages are in contrast to the biodegradation of the substrate supplied.

The results obtained show a similar trend than the results obtained with the application of RBCOD (readily biodegradable COD) methodology reported by [18]; in fact, also in this case the RBCOD/COD ratio decrease with the increase of substrate volume (Table 3).

Table 3

Results of the tests for the evaluation of RBCOD.

Tests	COD tested	RBCOD	RBCOD/COD
	[mg L^{-1}]	[mg L^{-1}]	[%]
RBCOD 1	2.0	247	**15**
RBCOD 2	4.0	150	**9**
RBCOD 3	32.1	130	**8**

The results obtained with the proposed methodology are significantly different than the RBCOD method ones, because the duration of the tests carried out in this work is higher with respect to RBCOD tests; moreover, the tests duration is similar than the HRT of WWTP that receive the substrate analyzed.

The proposed method allows to estimate the increasing of oxygen consumption in the WWTP biological reactor related to the treatment of aqueous waste pre-treated by AWTP. In this specific case, the results lead to an increase in OD needed of 35-40% in relation to COD fed (14 mg per liter of aqueous waste).

Finally, for each respirometric test, the nitrification activity of biomass was evaluated. In particular, it was found that consumed oxygen was not used for nitrification activity by the autotrophic bacteria, as the ammonia nitrogen average was below 0.08 mg L^{-1}, both at the beginning and at the end of test, and nitric nitrogen varied on average equal from 22 mg L^{-1} to 24 mg L^{-1} at the end of respirometric test. This provides that DO is consumed only through the biodegradation of organic substance.

4. Conclusions

The alternative method for the evaluation of ABS can simulate biologically oxidation treatment at the laboratory scale and allows determining the actual biodegradable fraction of organic substrate by the mesophilic biomass, by determining the kinetics of DO uptake rate. The analysis campaign results show that:

- ABS concentration varies, and in particular decreases with increasing of substrate added to respirometric test; this indicates that the biodegradable fraction depends on the operating conditions of biological reactor, such as the retention time inside the bioreactor and the COD fed; the increase in substrate added to respirometer involved a radical reduction of the actually biodegradable organic substrate;
- ABS concentration is not comparable to the BOD concentration obtained with the traditional method, because this last measure does not take into account the actual conditions occurring within the biological reactor.

In order to optimize the management of WWTP, the alternative method to the evaluation of ABS allows to:

- quantify the organic fraction (in a substrate) actually available to the biological process under the real operating conditions;
- evaluate the possible toxic-inhibition effect of a substrate to the biological process;
- determine the actual DO consumption required for substrate biodegradation within the biological reactor, both aqueous waste and urban wastewater.

Finally, this alternative method does not require any specific laboratory equipment, except the one for respirometric test performing, and provides results in a short time.

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