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Respirometry tests in wastewater treatment: why and how? A critical review

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Abstract

Respirometry tests are a widely employed method in the wastewater treatment field to characterize wastewater streams, assess toxic/inhibitory effects to the biomass, calibrate mathematical models. Respirometry can allow to fractionation the chemical oxygen demand (COD) in biodegradable and inert fractions, but also provide information related to biomass kinetics and stoichiometry through standardized laboratory techniques. Considering the increasing number of emerging contaminants detected in wastewater effluents, such as pharmaceuticals, personal care products and pesticides, respirometry can be a useful tool to promptly assess any toxic or inhibitory effect in wastewater treatment plant (WWTP) operations. Beside conventional activated sludge, in recent years respirometric methods have been applied to innovative fields, such as moving-bed bio-reactors (MBBRs), fungi and microalgae, exploiting natural remediation methods. In particular, respirometry application to microalgae, through the so-called photo-respirometry, has been investigated in the latest years in the treatment of high-loaded streams, allowing resource recovery in biomass form. In this work, respirometric methods are first introduced from a theoretical basis and then critically discussed by considering the experimental apparatus, the available characterization protocols and the fields of application; the most recent literature findings on respirometry are coupled with authors' experience in the field. A comparison between physicochemical methods and respirometry is made. The future research needed on the topic is finally outlined, including the coupling of respirometry with microbial community analysis, potentially leading to an enhanced process understanding, an extended respirometry utilization to get specific kinetic and stoichiometric parameters for modelling purposes, and a wider respirometry application as a diagnosis tool in WWTP operations.

Keywords: respirometry; wastewater treatment; mathematical modelling; activated sludge; microalgae; toxicity assessment.

1. Introduction

The biological treatment is the core section of wastewater treatment plants (WWTPs), and thus a detailed process knowledge is fundamental to allow smooth and efficient operations. Moreover, most of the electricity consumption for WWTP operations (even >50% in the case of innovative processes, such as membrane bioreactors, MBRs) is due to biological treatment for biomass aeration and sludge circulation (Egbuikwem et al., 2020), and consequently its optimization is crucial to enhance the overall WWTP energy efficiency. Traditionally, in municipal WWTPs the secondary biological treatment has been performed through conventional activated sludge (CAS), even if alternative solutions (including MBRs, sequencing batch reactors, SBRs, or microalgae) are available nowadays on the market (Nancharaiah and Sarvajith, 2019). .

Beside monitoring biomass activity and composition, the detailed characterization of wastewater streams is fundamental for efficient and smooth WWTP operations. Respirometry is generally defined as the measurement and interpretation of the biological consumption rate of an inorganic electron acceptor under well-defined experimental conditions (Spanjers and Vanrolleghem, 2016). Respirometry methods aim to evaluate the rapidly and slowly biodegradable COD (Chemical Oxygen Demand) fractions in wastewater streams, respectively called rbCOD and sbCOD (Zhang et al., 2021), but also to assess biomass kinetics and stoichiometry.

Traditional WWTP perspective, aimed at ensuring the required effluent quality by legislation limits, is currently shifting towards Water Resource Recovery Facilities (WRRFs) paradigm at all levels (Solon et al., 2019). In this new approach, resource and energy recovery is strongly incentivized, and mathematical models are a powerful tool to stimulate WWTPs transition into WRRFs, maximizing the value of the recovered products (Solon et al., 2019) and improving the energy and economic balance.

Activated sludge models (ASMs), in particular, developed by International Water Association (IWA), are a valuable design, simulation, control and optimization tool for biological WWTPs (Borzooei et al., 2021). However, they require a detailed wastewater characterization, being a challenge for their widespread application (Choubert et al., 2013), mostly considering that not all the required kinetic and stoichiometric parameters are commonly measured by water utilities. For a proper model application, in fact, conventional characterization parameters such as COD and nutrients (N, P) have to be fractioned into soluble and particulate state variables (Borzooei et al., 2021).

The need for a specific COD fractionation and a detailed kinetic and stoichiometric biomass analysis is particularly true when dealing with Advanced Oxidation Processes (AOPs), including sonication (Mainardis et al., 2019), ozonation (Mainardis et al., 2020), Fenton (Ma et al., 2021), photo-catalytic oxidation (Ma et al., 2021), electrochemical oxidation (Ma et al., 2021), aimed at treating refractory and poorly biodegradable streams. Respirometry has demonstrated to be extremely useful to get kinetic and stoichiometric parameters referred to the peculiar biomass and wastewater samples, rather than using default literature values (Mainardis et al., 2020).

Beside conventional wastewater treatment processes, microalgae, as a green and low-cost remediation technology, recently demonstrated a better capability of removing micropollutants when compared to CAS, particularly when considering recalcitrant substances such as pesticides (Liu et al., 2021). Thanks to their high versatility, microalgae can grow in a broad spectrum of wastewater streams, converting nitrogen and phosphorous into valuable products with a significant commercial value (Lutzu et al., 2021). Furthermore, microalgae-based remediation treatment can lead to the production of bioplastics, potentially replacing fossil fuels through third-generation biofuel generation (Callegari et al., 2020) Microalgae can be used as well for biogas production, leading to a full exploitation of circular economy principles and showing, in addition, a positive energy outcome (Carrillo-Reyes et al., 2021). Respirometry, specifically applied to microalgae (through the so-called photo-respirometry), is being used in an increasing number of applications to assess the specific activity of the tested algae strains.

Nowadays, particular attention is being given to emerging (or new generation) contaminants in the wastewater treatment sector, due to their widespread use and detection in several streams (Dhangar and Kumar, 2020). Emerging contaminants include a variety of compounds ranging from pharmaceuticals and personal care products (PCP) to flame retardants and antibiotic resistant genes (ARG) (Meng et al., 2021). Micropollutants of emerging concern can negatively affect biological process performances (Vasiliadou et al., 2018), as they are not efficiently removed, often requiring a tertiary treatment, mostly if the treated wastewater has to be reused in agriculture (Rossi et al., 2021) or industry (Nadeem et al., 2019). In addition, biotransformation processes in WWTPs can lead to a partial degradation of these compounds: consequently, the ecological toxicity to the receiving environment has to be specifically assessed (Nguyen et al., 2021). Respirometry is a useful technique to assess toxic or inhibitory effects posed by conventional and emerging contaminants to the biomass, leading to a fast detection of inhibitory phenomena in WWTPs, avoiding reactor failure. However, from the analysis of existing literature a lack of standardization emerges in the applied protocols and experimental apparatus used for respirometric tests; moreover, according to our knowledge, at present the potential of this technique in its diverse fields of application (toxicity assessment, wastewater and biomass characterization, mathematical modelling, photo-respirometry) is not fully exploited by researchers and water utilities. At the best of our knowledge, despite the high number of respirometry applications in the recent scientific literature, a thorough review on the topic is still missing.

The present paper is aimed at giving an up-to-date vision of respirometry applications and recent developments, analyzing the state-of-the-art and pointing out the critical points to be deepened in the near future. Respirometry tests will be first reviewed from a theoretical basis (Section 2). The commonly used laboratory apparatus and the innovative technological devices will be described in Section 3, while Section 4 will present the available literature protocols for respirometric characterization. The main applications of respirometric techniques (including toxicity and inhibition assessment, wastewater and biomass characterization, mathematical modelling, microalgae) are summarized in Section 5, together with an outlook on the future research needed on the topic.

2. Respirometry: theoretical basis

As previously introduced, respirometry is a biological method widely used in wastewater COD characterization (Zhang et al., 2021) especially for activated sludge systems (Karlikanovaite-Balikci and Yagci, 2019), and generally involves measuring the rate at which the biomass assimilates a substance from the liquid or produces a component (Spanjers and Vanrolleghem, 2016). The experimental equipment for respirometry ranges from simple, manually operated bottles equipped with sensors, up to sophisticated instruments able to operate automatically (Caffaz et al., 2007).

The most commonly adopted classification of respirometer systems is reported in Fig. S1. A basic distinction can be made between the phase where the concentration is measured (i.e., gas or liquid) and the presence or absence of gas and liquid flows in the reactor (Rossi et al., 2020a). Liquid phase, static gas, static liquid (LSS) respirometer is the simplest device because of the absence of liquid or gaseous flows (and also of pumps for aeration); Biochemical Oxygen Demand (BOD) measurement is an example of practical LSS application (Borzooei et al., 2021). Alternatively, gas phase, static gas, static liquid (GSS) respirometers require a relation between the measured dynamics in the gaseous phase and the respiration rate in the liquid phase (Spanjers and Vanrolleghem, 2016).

The simplest respirometers used in laboratory investigations are based on aqueous dissolved oxygen (DO) monitoring, and calculate DO mass balance over the liquid phase (Rossi et al., 2020a). The general equation describing DO mass balance can be written as follows, where Q_{in} and Q_{out} (L/h) are respectively the liquid flowrates entering and leaving the system, while $S_{O2,in}$, S_{O2} and S_{O2}^{*} are DO concentrations respectively in the liquid phase entering the system, at a generic time *t* and at saturation conditions (mg/L), V_L is the liquid volume (L), $k_{L,a}$ is the oxygen mass transfer coefficient (1/h) and r_{O2} represents biomass respiration rate in the liquid phase (mg/L h) (Spanjers and Vanrolleghem, 2016):

$$\frac{d(V_L S_{O_2})}{dt} = Q_{in} S_{O_2,in} - Q_{out} S_{O_2} + V_L k_{L,a}\left(S_{O_2}^{*} - S_{O_2}\right) - V_L r_{O_2} \tag{1}$$

In the simplest case, where both gas and liquid phases are static, the mass balance reduces to:

$$\frac{dS_{O_2}}{dt} = -r_{O_2} \tag{2}$$

In this case, to obtain the respiration rate it is sufficient to measure DO concentration throughout time. Moreover, no mass transfer is considered from the gas to the liquid phase, according to what proposed in the Standard Methods for Examination of Water and Wastewater (APHA, 2012).

One of the basic respirometry applications involves the evaluation of the readily biodegradable COD (rbCOD, or S_S) fraction in a generic wastewater stream through the so-called deoxygenation tests. Normally, when a biomass has to be tested in a respirometric apparatus, it is recommended to continuously aerate the sludge for about 24 h after its withdrawal to establish fully endogenous conditions (i.e., no external substrate is available for the sludge) (Mainardis et al., 2020).

The oxygen consumption due to the exogenous respiration is monitored in the deoxygenation test. A defined volume of activated sludge is put in a thermostatic (18-20 °C) vessel and is aerated until a stable state is reached (corresponding to saturation conditions) (Mainardis et al., 2020). Acetate is commonly utilized to simulate a readily biodegradable carbonaceous substrate (Arias-Navarro et al., 2019), while ammonium chloride can be used to evaluate autotrophic respiration rate. Nitrate Uptake Rate (NUR) can be studied as well by means of respirometry (Vitanza et al., 2016). If only the degradation of the organic fraction is considered, a nitrification inhibitor (such as allylthiourea) has to be added to the reactor (Borzooei et al., 2021).

The deoxygenation tests forecast adding different amounts of substrate to the biomass and graphically evaluating exogenous DO consumption, by plotting the DO behavior versus time (Fig. 1a). The oxygen uptake rate (OUR) curve, simply obtained through calculating the derivative of the DO curve, is normally depicted as well, to assess the maximum OUR (in exogenous conditions, OUR_{ex}) and the baseline OUR (due to endogenous respiration, OUR_{end}) (Fig. 1b). From OUR (generally expressed as mg O_2/L h), it is also possible to get the specific oxygen uptake rate (SOUR, mg O_2/g h), calculated through Eq. 3, where MLVSS (g/L) is the concentration of mixed liquor volatile suspended solids of the tested biomass (Arias-Navarro et al., 2019).

$$\mathrm{SOUR} = \frac{\mathrm{OUR}}{\mathrm{MLVSS}} \tag{3}$$

The behavior of OUR throughout time is generically called a respirogram. The dosage of a readily biodegradable substrate (rbCOD, or S_S) to a sludge in an endogenous phase leads to an abrupt increase in OUR, (expressed in Fig. 1b as mg O_2/L min) until a maximum value is reached, determined by biomass activity and substrate degradation rates (Spanjers and Vanrolleghem, 2016). Finally, the added substrate gets exhausted, and the endogenous respiration rate is reached again. The exogenous oxygen consumption obtained from the different tested substrate concentrations is then summarized in a calibration line (Fig. 1c). A further deoxygenation test is successively conducted with the actual wastewater sample to be tested, and the exogenous oxygen consumption is converted to acetate COD fraction ($COD_{ww,ac}$, mg COD/L) by using the calibration line. Finally, the readily biodegradable COD fraction (S_S or rbCOD) in the tested wastewater is calculated by considering the sludge (V_{slud}, L) and wastewater (V_{ww}, L) volumes used in the deoxygenation test (Eq. 4).

$$\mathrm{S_S} = \mathrm{COD_{ww,ac}} \frac{\mathrm{V_{slud}} + \mathrm{V_{ww}}}{\mathrm{V_{ww}}} \tag{4}$$

For each deoxygenation test, it is also possible to calculate the heterotrophic biomass yield (Y_H, mg COD biomass/mg COD substrate) (Eq. 5) by considering the exogenous oxygen consumption (ΔDO, mg O_2/L, Fig. 1a) and the substrate dosage (COD_{ac}, mg COD/L).

$$\mathrm{Y_H} = 1 - \frac{\Delta \mathrm{O_2}}{\mathrm{COD_{ac}}} \tag{5}$$

FIGURE 1

Longer respirometry tests involve, as an example, the 24-h respirogram test with OUR analysis, that is used to evaluate the total biodegradable COD fraction, composed of readily and slowly biodegradable compounds (respectively rbCOD and sbCOD, or S_S and X_S). These tests require a significantly higher substrate dosage, when compared to deoxygenation tests, due to the longer duration. In 24-h tests, a proper aeration regime (typically intermittent mode) has to be selected, and wastewater dosage must be carefully calculated considering F_0/X_0 ratio (i.e., the relative ratio between initial substrate and biomass concentrations, expressed as mg COD/mg VSS). In Eq. 6, COD_{ww} is the COD concentration in wastewater (mg COD/L).

$$\frac{F_0}{X_0} = \frac{COD_{ww} \cdot V_{ww}}{MLVSS \cdot V_{slud}} \tag{6}$$

In literature, the optimum F_0/X_0 ratio is reported to be in the range of 0.01-0.05 mg COD/mg VSS; excessive F_0/X_0 values can lead to an unwanted microorganism growth, with temporal changes, while a very low F_0/X_0 ratio causes a fast substrate assimilation, difficult to monitor if the measuring device is not properly set (Borzooei et al., 2021). Regarding the test outcomes, the OUR curve is again used as the main indicator to calculate the overall biodegradable COD fraction (composed of $S_S + X_S$).

FIGURE 2

An example of OUR curve obtained from these tests is reported in Fig. 2, where the exogenous and endogenous oxygen consumptions are highlighted. After the initial OUR peak, due to rbCOD degradation, a long tail is frequently observed, due to sbCOD conversion, until endogenous conditions are finally restored (Spanjers and Vanrolleghem, 2016). The total COD biodegradable fraction, composed of fast (rbCOD or S_S) and slowly (sbCOD or X_S) degradable organic matter, is finally calculated through Eq. 7, by considering sludge and wastewater volumes (known), Y_H (obtained from deoxygenation tests), and exogenous oxygen consumption, ΔDO (mg O_2/L).

$$X_S + S_S = \frac{\Delta DO}{1 - Y_H} \frac{V_{slud} + V_{ww}}{V_{ww}} \tag{7}$$

3. Experimental apparatus

The basic equipment needed to conduct respirometry assays consists of a stirred vessel (operating volume from a few 100 mL up to several liters), where biomass and substrate are combined (Borzooei et al., 2021; Faria et al., 2021), a mixing system, and an aerator that provides the air for sustaining the aerobic respiration (Fig. S2). A DO probe is inserted in the reactor; data handling can be either manual or automatic. An analytical algorithm able to determine Monod kinetic parameters was recently developed to be used in modern programmable respirometers (Wu and Chiang, 2020); the sensitivity analysis demonstrated that it is essential to use relatively high initial substrate levels (F_0/X_0 ratio) and properly acclimated seed cultures to get reliable results. The data acquisition from the DO sensor has to be fast enough to correctly follow the biochemical process kinetics (Spanjers and Vanrolleghem, 2016).

Basing on the measuring principle, the vessel can be completely sealed (to prevent oxygen exchange with the gaseous phase) or open (Spanjers and Vanrolleghem, 2016). Normally, in laboratory tests the reactors are immersed in a water bath to control the operating temperature (optimal values of 18-20 °C). The main advantage of applying respirometric techniques, when compared to traditional BOD measurement, is the possibility to get OUR profile throughout time, leading to the determination of important kinetic parameters (Rahman and Islam, 2015).

FIGURE 1

Beside traditional respirometric systems, newly devices have been developed in literature to cope with particular applications (Table S1). Significant attention has been given to micro-respirometry, that combines classical respirometry methods with micro-reactors for the characterization of wastewater microbial cultures, leading to a reduced operating volume, together with a simplification of model calibration due to the higher number of experimental data (Vital-Jacome et al., 2017). The micro-respirometric reactors have a very small volume (down to 4 mL) and normally do not forecast any airflow, exploiting only superficial aeration; a pulse dynamic protocol can be employed, including DO measurement after substrate pulse injection at known concentration (Lu et al., 2020).

A part from CAS, respirometry has been shown to be useful in simulating also moving-bed bio-reactors (MBBRs) (Bouteraa et al., 2019; Ferrai et al., 2010): the assessment of OUR profiles in MBBR biofilm samples can provide valuable information for mathematical simulations. Conventional respirometric protocols were commonly applied in literature to the detached MBBR biomass; the results highlighted a strong biomass propensity to accumulate substrate and a lower value of maximum specific heterotrophic growth when compared to default literature values applied for CAS modelling (Henze et al., 2000).

In the case of fixed-bed reactors, instead, it was shown that maintaining biofilm integrity was crucial for the kinetic tests; respirometry was proved to be ideal for a regular biofilm kinetics measurement and optimization (Lu et al., 2020; Ordaz et al., 2019).

Fungi are another technology being recently investigated in literature (Caffaz et al., 2007), particularly in the treatment of tannery wastewater, since tannins are poorly removed through CAS or require costly physicochemical treatments (Singh et al., 2020). Moreover, several fungi are able to exploit tannins as energy sources. Even if long-term operations of fungal-based bioreactors is challenging (Bardi et al., 2017), recently a laboratory scale reactor for tannery wastewater remediation based on fungi (growing in polyurethane foam cubes) was successfully tested under non-sterile conditions (Spennati et al., 2020). Pulse-flow respirometry (i.e., utilization of multiple pulses of substrate and OUR analysis to detect kinetic and stoichiometric parameters) was employed to assess relevant kinetic and stoichiometric parameters for modelling purposes (Table S1).

4. Respirometric protocols

A limited number of respirometric protocols is available in the scientific literature; however, most of them are related to long-term biodegradability evaluations (such as BOD measurement), and thus cannot be efficiently used to promptly detect biomass kinetic and stoichiometric parameters, as well as operational issues in WWTP managing operations. As an example, the protocol OECD 301F (proposed by the Organization for Economic Co-operation and Development), generically aims at evaluating substrate biodegradability in aqueous media. The method involves adding 100 mg/L of substrate as sole carbon source in a closed reactor at constant temperature, and requires DO monitoring throughout 28 days of tests (Organisation for Economic Co-operation and Development, 1992). Similarly, the Standard Methods for Examination of Water and Wastewater mention in Section 5210D the respirometric methods as a useful technique for chemical biodegradation, toxicity/inhibition assessment, analysis of oxidation rates. Four types of commercial respirometers are mentioned: manometric (constant volume, pressure change), volumetric (constant pressure, volume change), electrolytic, direct-input respirometers. However, a standardized procedure is not defined in the text, because of the recognized differences between uses, seed cultures, instruments, and results applicability. Thus, only some general recommendations and guidelines are suggested (APHA, 2012).

In 2010, OECD proposed in its document OECD 209 a further guideline for assessing acute toxicity effects (Organization for Economic Co-operation and Development, 2010): following this method, the measurement of the respiration rate has to be conducted under different concentrations of the tested toxicant, providing a fast procedure to assess toxic or inhibitory effects on activated sludge microorganisms. The results are normally expressed in terms of IC_{50} (50% inhibitory concentration), that represents substance concentration that reduces the respiration rate by 50%.

Other guidelines, more specifically aimed at calibrating WWTP mathematical models, mention the possibility to apply respirometric tests to get “real” kinetic and stoichiometric coefficients. However, in most cases the modelling protocols do not analytically describe in detail the respirometric procedures and, in addition, they do not agree on the best characterization methods to use (Borzooei et al., 2021). As an example, while the STOWA protocol (Hulsbeek et al., 2002) is focused on physicochemical characterization and long-term bioassays, the BIOMATH protocol (Vanrolleghem et al., 2003) is based on respirometry. WERF protocol (Melcer, 2004), instead, suggests the combined utilization of both respirometric and physicochemical approaches. Consequently, a lack of standardization emerges from the analysis of the existing literature; in addition, often the respirometry guidelines are not properly updated to cope with new applications, such as micro-respirometry, fungi, microalgae.

5. Applications

Section 5.1 describes respirometry application to assess toxic or inhibitory effects to the biomass, while Section 5.2 is referred to the characterization of wastewater and biomass. Section 5.3 deals with respirometry application for the calibration of mathematical models; Section 5.4, instead, is focused on photo-respirometry (i.e., respirometry application to microalgae). The advantages and drawbacks of respirometry are briefly summarized in Section 5.5, while Section 5.6 depicts future research needed on the topic.

5.1 Toxicity and inhibition

One of the most common respirometry applications is the assessment of toxic or inhibitory effects, given by conventional or emerging pollutants, to the biomass. Inhibition is defined as a reversible reduction in the normal biological functions, while toxicity is related to an adverse effect on the biological metabolism, and is commonly irreversible (Spanjers and Vanrolleghem, 2016). Often, the results of toxicity tests are expressed as IC_{50} (50% inhibition concentration), which is the agent’s concentration that produces a reduction of 50% in the biological respiration rate (Spanjers and Vanrolleghem, 2016). In literature, respirometry has been applied to assess toxicity effects to both nitrifying and heterotrophic bacteria, and these tests are generally based on OUR evaluation in presence and absence of toxicants (Ren, 2004). Toxicity effects can be assessed by evaluating the percent reduction in the maximum exogenous oxygen consumption rate ($r^{max}_{O2,exo}$) before and after the addition of the toxicant (Eq. 8); however, also endogenous respiration rate can be used to this purpose (Spanjers and Vanrolleghem, 2016).

$$\text{Toxicity (\%)} = \frac{r^{max}_{O2,exo}(\text{before}) - r^{max}_{O2,exo}(\text{after})}{r^{max}_{O2,exo}(\text{before})} \cdot 100 \tag{8}$$

A meaningful example related to inhibition effects to ammonia-oxidizing bacteria (AOB) and nitrate-oxidizing bacteria (NOB) given by amoxicillin (a common pharmaceutical) (Fig. 3a-b) shows that the evaluation of exogenous respiration rate gives a clearer indication than endogenous respiration (Faria et al., 2021); consequently, it is recommended to focus on the former, when dealing with biomass inhibition, particularly if the endogenous OUR is low in the baseline scenario.

FIGURE 3

The most recent literature studies focused on respirometry application to evaluate toxic or inhibitory effects are summarized in Table 1. Most of the reported studies dealt with acute (i.e., short-term) toxicity (Aguilar et al., 2020; Cristóvão et al., 2016; Ortiz de García et al., 2014; Tominaga et al., 2018): as an example, (Faria et al., 2021) studied toxicity cycles lasting 8 h through respirometry. However, some remarkable study (Vasiliadou et al., 2018) investigated also long-term toxicity effects, giving useful insights on the possible issues related to a long-term reduction in sludge activity that could be observed in full-scale WWTP operations. Respirometric tests were shown to be useful in evaluating the toxicity of a wide spectrum of contaminants of emerging concern (CECs), such as pharmaceuticals, personal care products, heavy metals, nanoparticles.

TABLE 1

Salinity, in addition, is a relevant parameter in wastewater treatment, considering that excessive salinity concentration is known to potentially impact in a negative way the performances of biological remediation processes; some remarkable studies (Cristóvão et al., 2016; Mannina et al., 2016) investigated saline wastewater treatment (including streams produced by fish industry), analyzing the stress effects given to the biomass and the inhibitory levels.

5.2 Wastewater and biomass characterization

COD can be classified, according to the particle size, in suspended solids (diameter>450 nm), colloids (1 nm<diameter<100 nm) and dissolved substances (diameter<2 nm) (Wei et al., 2019). The most common method to characterize COD in wastewater treatment is membrane filtration with pore diameters of 0.45 µm (Wei et al., 2019). This basic physical distinction between soluble (S) and particulate (X) COD can be further deepened by assessing the biodegradable (S_S, X_S) and inert (S_I, X_I) fractions (according to substrate biodegradability, Fig. 4). In wastewater COD fractionation, for simplicity reasons normally autotrophic and heterotrophic biomass concentration is neglected.

FIGURE 4

Respirometric techniques can allow determining the biodegradability of a wide range of municipal and industrial wastewater streams, leading to a complete COD fractionation. Respirometry has been applied in the recent years to investigate the biodegradability of various substances, from single compounds to complex wastewater streams (Table 2).

TABLE 2

Beside industrial streams, respirometry applicability to municipal wastewater has been confirmed as well, with some limitations when dealing with extremely diluted effluents. Regarding the biomass, activated sludge treating carbonaceous substrates is primarily composed of heterotrophic biomass and endogenous residues; also, extracellular polymeric substances (EPS) account for a portion of the MLVSS (Ramdani et al., 2012). The estimation of heterotrophic biomass activity is essential for the design and operation of WWTPs: remarkable literature studies were aimed at evaluating the differences between adapted and raw biomass (in the treatment of complex industrial streams) (Corsino et al., 2020) and at assessing the activity of different bacterial populations (such as hydrolytic bacteria) (Benneouala et al., 2017).

Some innovative approaches proposed in the investigate literature (Ke et al., 2015; Raper et al., 2019) combine respirometry and microbial community analysis, considering that microbial community structure and diversity significantly impact on WWTP performances and stability (Yang et al., 2020); the outcomes of respirometry tests can thus be effectively supported by biomolecular techniques. Microbial community analysis enables researchers to assess the composition of bacterial populations, connecting them to process performances (Karlikanovaite-Balikci et al., 2019). Bacterial richness and diversity depend not only on the adopted wastewater treatment processes, but also on influent wastewater characteristics: as an example, it was recently shown that chemical industrial wastewater inhibits microbial community diversity and richness, while these indicators are positively correlated with conventional macropollutants (i.e., carbon, nitrogen, phosphorus) (Yang et al., 2020). More in detail, high-throughput sequencing techniques, such as 454 pyrosequencing and Illumina sequencing platforms, enable the determination of complex microbial communities and different microbial pathways with enough sequencing depth and high accuracy (Yang et al., 2020).

5.3 Calibration of mathematical models

Different mathematical models have been built and updated in recent decades for simulating WWTP operations and analyzing alternative scenario for process optimization. Among them, activated sludge models (ASMs), developed by International Water Association (IWA), are widely used by researchers and water utilities to improve process management and reduce energy consumption in WWTPs (Martin and Vanrolleghem, 2014). Respirometry techniques allow to get specific kinetic and stoichiometric parameters on the tested biomass, beside simply affording on literature and software default values, and thus are fundamental to properly calibrate WWTP mathematical models.

The reported literature studies related to respirometry application for modelling purposes are summarized in Table 3.

TABLE 3

Remarkably, this technique has been shown to be affordable at different scales, including laboratory, pilot, and full-scale investigations. Reported reactor configurations include a wide range of processes, such as CAS, oxidation ditches, MBRs. One of the main aims of respirometry application to WWTP simulation is the optimization of plant operating parameters (e.g., Hydraulic Retention Time, HRT, Solid Retention Time, SRT, recycle ratios), that can lead to smoother operations with relevant economic savings. Respirometry can also be considered as a diagnosis tool to detect and solve operational issues linked to the specific plant configuration, preventing possible faults in WWTP operations (Arias-Navarro et al., 2019).

Some remarkable studies, in addition, investigated respirometry application to evaluate the reduction in heterotrophic growth (and consequently excess sludge production) after introducing innovative sludge treatment techniques, such as ozonation (Gardoni et al., 2011) and anaerobic side-stream reactors (ASSR) (Velho et al., 2019). The overall reduction of excess sludge production is an extremely hot topic in the literature, considering that nowadays sludge management and treatment represents a significant share of the operating costs (>30%) for water utilities (Khakbaz et al., 2020).

5.4 Microalgae

Microalgae are a promising and sustainable alternative to traditional wastewater remediation technologies; through microalgae treatment, the nutrients present in wastewater streams (particularly in highly-concentrated effluents) are efficiently exploited to produce biomass (Sforza et al., 2018) that can be successively used in a pool of valuable applications (Hussain et al., 2021). Microalgae having short life span, high growth rate and consistent CO_2 utilization efficiency are particularly indicated to produce renewable resources from wastewater (Hussain et al., 2021). The utilization of phototrophic organisms, such as microalgae, is gaining momentum in the wastewater treatment sector, due to their capability of exploiting photosynthetic oxygenation to reduce the operating costs and the environmental impacts (Rossi et al., 2020a).

Properly set respirometric methods can be employed to assess the photosynthetic activity of phototrophic organisms, efficiently monitoring algae-based wastewater treatment systems, and calibrating the related mathematical models. Photo-respirometry has been investigated in the recent literature to allow the application of properly modified respirometric methods to microalgae (Table 4). Differently from traditional biological processes, in microalgae treatment there exist two opposite oxygen fluxes, as O_2 is both generated as photo-synthesis by-product and consumed through the algal respiration (Rossi et al., 2020a). Consequently, both oxygen production rate (OPR) and OUR are determined in photo-respirometry tests.

TABLE 4

Regarding the respirometric equipment, in photo-respirometry tests it is fundamental for microalgae growth to have a light source, able to reach the desired irradiance in the reactors. Temperature, pH and DO are other fundamental process parameters that greatly influence the results (Rossi et al., 2020b). Furthermore, the respirometric vessels must be built using materials with high light transmittance (e.g., polycarbonate, glass, acrylic polymers) (Rossi et al., 2020a).

Through analysis of the specific oxygen uptake rate (SOUR) and the specific oxygen production rate (SOPR), it is possible to determine the phototrophic decay rate and the photosynthetic activity of algae and cyanobacteria (Tang et al., 2014). Compared to traditional growth studies, photo-respirometry allows a more rapid kinetics determination under different stress and environmental conditions (e.g., nutrients, pH, exposure to metals and chemicals) (Tang et al., 2014).

Recently, a series of guidelines was developed in the literature for photo-respirometry, specifically tailored for conducting respirometric tests on algal-bacterial suspensions, given the lack of official guidelines (Petrini et al., 2020; Rossi et al., 2020a, 2020b; Sánchez-Zurano et al., 2020); these indications can be included in the monitoring strategies of algal ponds or photobioreactors (Rossi et al., 2018). Alternating light/dark conditions and properly dosing substrates/inhibitors allow to selectively activate/inactivate specific microalgal or bacterial metabolisms, consequently determining their kinetics (Rossi et al., 2018). It should be highlighted that AS kinetics cannot be directly applied without modifications to algae photobioreactors, because of the important differences in composition, growth and decay rate. Regarding the investigated streams, most of the reported studies on photo-respirometry and microalgae treatment investigated high-strength effluents, such as digestate liquid fraction or blackwater, where microalgae can be particularly profitable, especially when compared to CAS.

5.5 Summary of respirometry pros and cons

The main pros and cons of respirometry tests, according to the analyzed literature evidences, are briefly summarized in Table S2. Many authors agree on the possibility to get several information about microbial populations (such as biomass kinetics and stoichiometry) through respirometry in a simple manner, but also to assess biodegradable COD fraction in municipal and industrial streams. The recognized downsides are mostly related to a reduced respirometry robustness when dealing with extremely diluted effluents and to a partial misalignment with physicochemical methods. According to authors' experience, in addition, respirometric tests can be extremely useful in the characterization of wastewater streams in different operating scenarios (i.e., dry and wet weather) in order to apply detailed WWTP process modelling. The experimental conditions of the laboratory tests, however, must be properly set to get robust results, as the obtained outcomes are strongly influenced not only by the peculiar wastewater characteristics, but also by sludge composition and activity. Consequently, a preliminary set of tests is required to fix the most important operating parameters in the respirometric bench device (such as biomass dilution, substrate dosage, substrate to biomass ratio, F_0/X_0, aeration and mixing intensity) before executing any respirometric campaign.

TABLE S2

As previously introduced (Section 4), several modelling protocols were proposed in the literature to simulate activated sludge process, either proposing respirometric methods or a combination of respirometry and physicochemical characterization as a reference tool. Physicochemical characterization is focused on filtration, that is influenced by pore size and sample pretreatment: soluble fractions are commonly determined at 0.45 µm or 0.1 µm pore size. Also, pre-flocculation followed by filtration at 0.45 µm can be performed, stimulating molecule aggregation and removal through successive filtration. Physicochemical methods, in addition, include BOD and COD analysis to get the overall COD fractionation.

The main difference between physicochemical and respirometric methods in COD fractionation is due to the fact that some substances, especially in industrial wastewater streams, despite passing through the filters are not easily biodegradable. Moreover, through physicochemical methods a larger X_S fraction can be detected, considering that the BOD tests have a longer duration than common respirometric assays (see Fig. 2). Thus, it is suggested to use both respirometric and physicochemical characterization methods to get a broader overview, especially when dealing with complex and poorly biodegradable industrial wastewater streams, where marked differences may arise between the two methods.

5.6 Future research needs

According to the conducted literature analysis and authors' expertise, future research that is needed to boost a wider respirometry integration in wastewater treatment operations should be focused on the following key areas:

- Development of more detailed laboratory protocols, focused on the specific process applications (i.e., CAS, MBBRs, microalgae, fungi etc.) to allow an easier comparison between the results obtained by different researchers;
- Wider respirometry integration with microbial community analysis, to better exploit new sequencing techniques and get useful indications for process optimization;
- Enhanced visibility and application of respirometry as a diagnosis tool in WWTPs, allowing a wider utilization by water utilities in WWTP management;
- Better integration of respirometry tests in the complex eco-toxicity assessments, particularly when considering emerging pollutants.

6. Conclusions

In this study, respirometric tests, including theoretical background, experimental apparatus, available protocols, field of applications and innovative technological solutions, were critically described and discussed by considering the most recent literature outcomes and authors' experience in the field. It was shown that these assays can be useful to quickly determine toxic or inhibitory effects to the biomass, particularly from emerging and refractory pollutants. Moreover, respirometric tests allow to fraction the influent COD in wastewater streams in biodegradable and inert compounds, giving useful insights for the calibration of mathematical models through direct measurement of relevant kinetic and stoichiometric parameters. In addition, respirometry is being currently investigated in the microalgae sector (through the so-called photo-respirometry) as a promising approach to evaluate algae strains activity in innovative wastewater remediation processes. Respirometric apparatus was properly adapted to investigate also MBBRs, fungi biomass and fixed-bed reactors. However, a lack of standardization in the applied protocols emerged from the literature analysis. Respirometry comparison with physicochemical methods highlighted the robustness of respirometry as a useful diagnosis tool for WWTPs to allow smooth operations; nonetheless, COD fractionation could highlight some differences between physicochemical characterization and respirometry when testing industrial streams. The coupling of respirometry and microbial community analysis is particularly promising to give further insights in the process dynamics, and could be a powerful tool for an enhanced respirometry application in the wastewater treatment sector.

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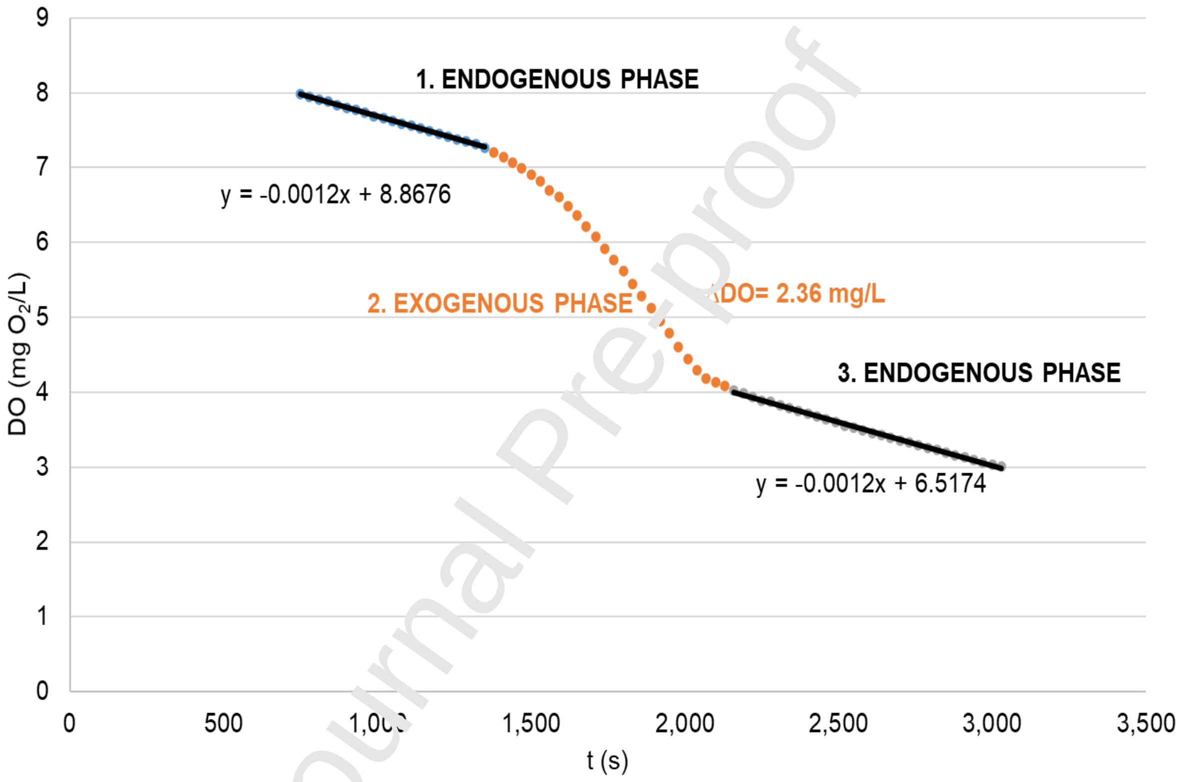
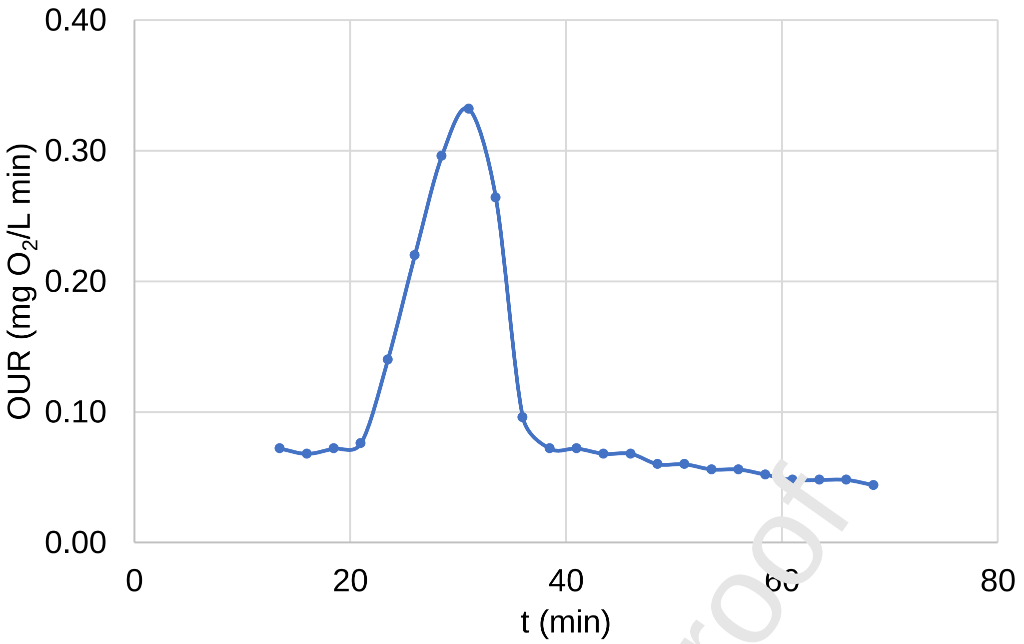
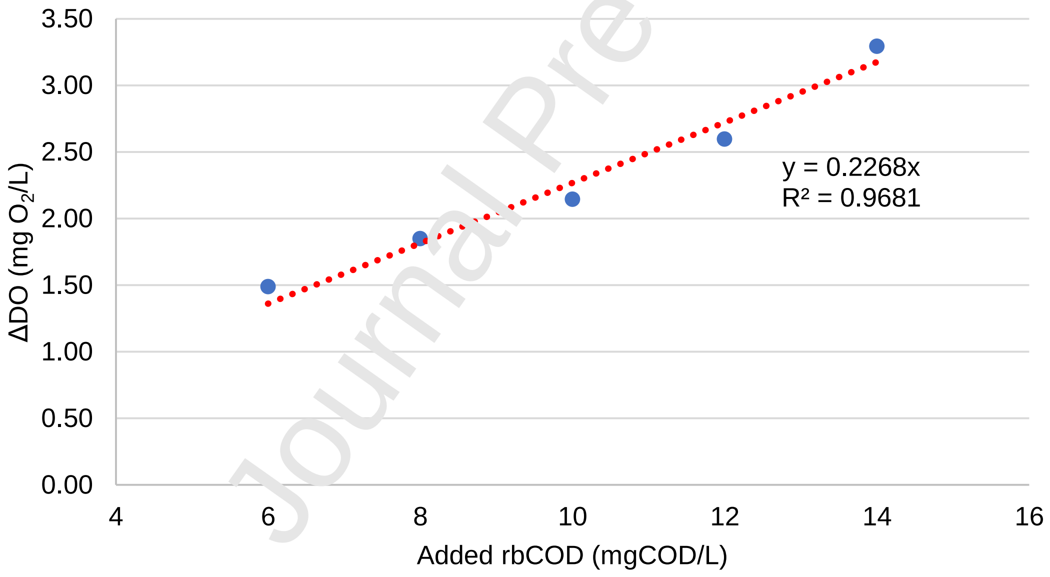
Fig. 1. Example of deoxygenation curve, reporting Dissolved Oxygen (DO, mg O_2/L) consumption throughout time (t, s) after adding 10 mg COD/L of acetate, with calculation of exogenous oxygen consumption (ΔDO, mg O_2/L) (Fig. 1a), the corresponding Oxygen Uptake Rate (OUR, mg O_2/L min) curve (Fig. 1b) and graphical representation of the calibration curve with acetate, reporting ΔDO versus the added readily biodegradable COD (rbCOD, mg COD/L) (Fig. 1c).



(a)


0.40
0.30
0.20
0.10
0.00
OUR (mg O_2/L min)
0
20
40
60
80
t (min)

(b)


3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
ΔDO (mg O_2/L)
4
6
8
10
12
14
16
Added rbCOD (mgCOD/L)
y = 0.2268x
R² = 0.9681

(c)

Fig. 2. Graphical example of calculation of the exogenous respiration area (ΔDO, mg O_2/L) from the 24-h Oxygen Uptake Rate (OUR, mg O_2/L min) curve.

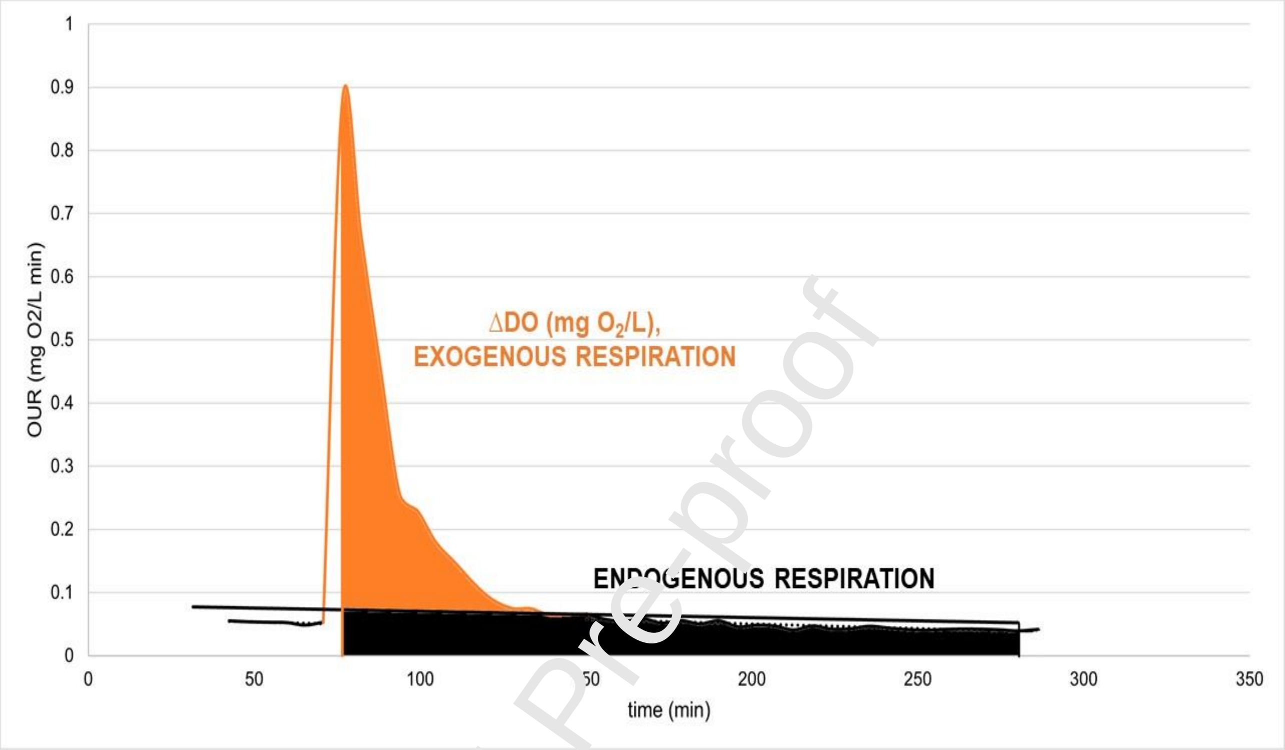
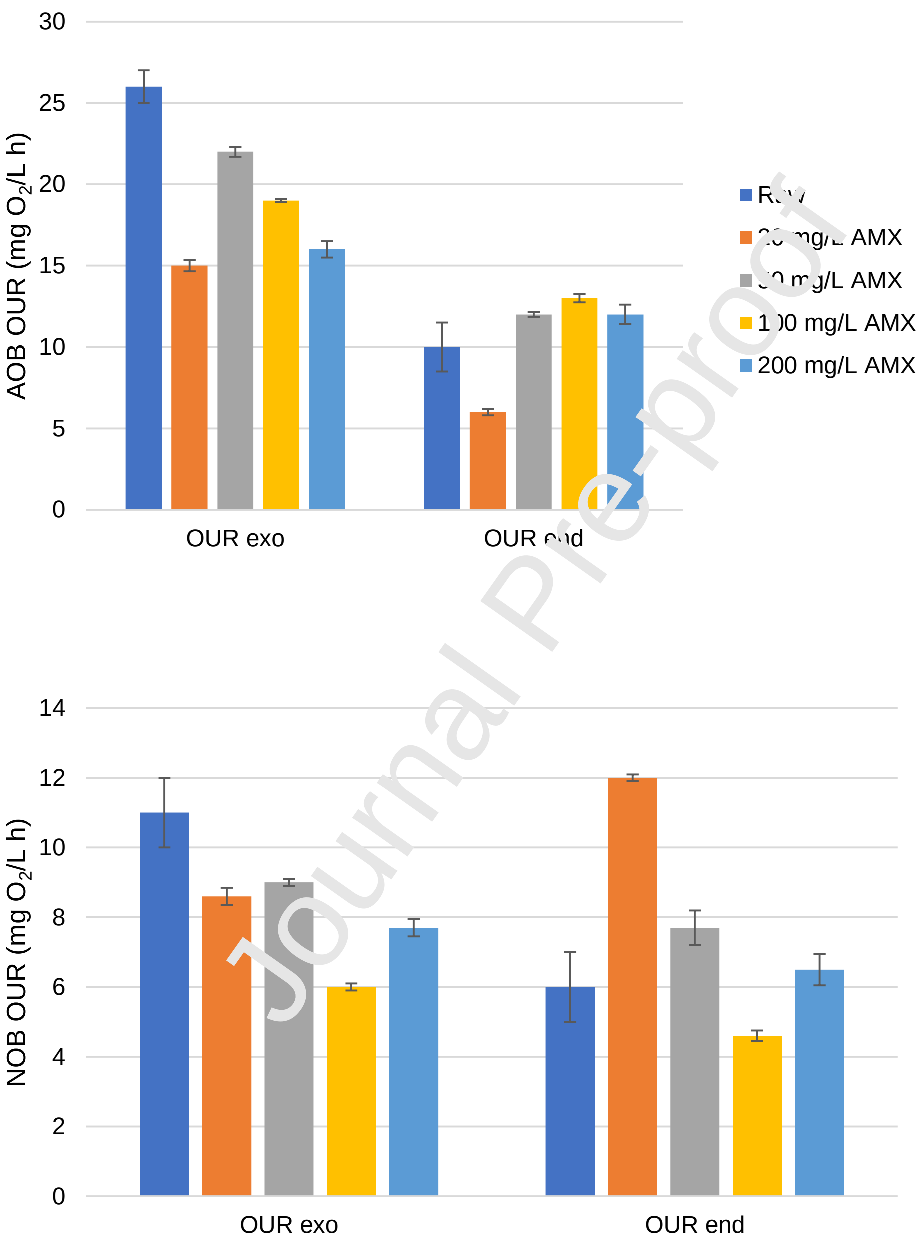


Fig. 3. Example of inhibitory effects given by amoxicillin (AMX) on exogenous (OUR_{exo}) and endogenous (OUR_{end}) oxygen consumption rate (OUR) (expressed as mg O_2/L h) of ammonia-oxidizing bacteria (AOB, Fig. 3a) and nitrite-oxidizing bacteria (NOB, Fig. 3b) (modified from (Faria et al., 2021))



a)

b)

Fig. 4. COD fractionation in wastewater, where tCOD indicates total COD, S and X are respectively the soluble and particulate fractions, while S_S, S_I, X_S, X_A, X_H and X_I indicate respectively the soluble biodegradable fraction, the soluble inert fraction, the particulate biodegradable fraction, the autotrophic biomass, the heterotrophic biomass and the particulate inert fraction.

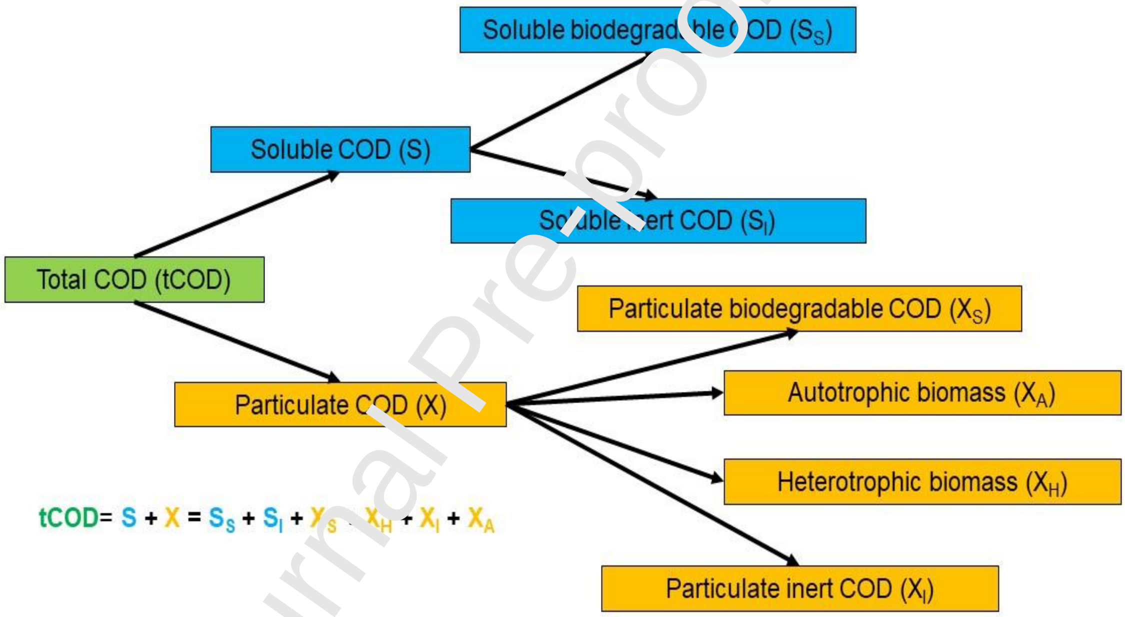


Table 1. Literature outcomes related to respirometry application to assess toxic/inhibitory effects.

Reactor	Aim of the work	Respirometry results	Reference
Pilot batch reactor	Assess amoxicillin effects on ammonia oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB)	Specific growth rate reduction (from 0.50 to 0.13 1/d for AOB and from 0.64 to 0.15 1/d for NOB), with severe limitations to nitrification	(Faria et al., 2021)

Liquid-flowing-static respirometer	Pharmaceuticals (caffeine, sulfamethoxazole, carbamazepine) effect on activated sludge activity	High toxicity posed on non-acclimated sludge; inhibition reduction in acclimated bacteria, with microbial community shift towards multi-resistant genera	(Vasiliadou et al., 2018)
Laboratory respirometer	Environmental risk assessment of 26 pharmaceuticals and personal care products (PPCPs)	Compounds' classification according to ecotoxicity outcomes; 65.4% of the investigated substances were highly toxic	(Ortiz de García et al., 2014)
Open semi-continuous respirometer	Diclofenac degradation through electron beam irradiation	Formation of recalcitrant and non-biodegradable products at high radiation doses	(Tominaga et al., 2018)
Liquid-flowing-static respirometer	Heavy metal (Hg, Zn, Cr, Pb, Ni) toxicity on activated sludge bacteria	Toxicity: Hg>>Zn>Cr>Pb>Ni; specific growth yield of 0.47-0.71 mg VSS/mg sCOD	(Aguilar et al., 2020)
Laboratory respirometer	Assessment of Cu^{2+} and Cu nanoparticles toxicity effects on activated sludge	10 mg/L of Cu^{2+} significantly reduced respiration rate (-55%), with toxicity for coliforms and AOB; Cu nanoparticles not inhibitory	(Ganesh et al., 2010)
Liquid-static-static respirometer	Effect of nanoparticles (cerium and titanium dioxide, silver, gold) on	Inhibitory effects: CeO_2>Ag>Au=TiO_2; anaerobic bacteria more easily inhibited	(García et al., 2012)

	wastewater treatment	than aerobic microorganisms	
Sequential batch membrane bio-reactor (MBR)	Assessment of salinity influence on carbon and nitrogen removal	Stress effect due to salinity, with respiration rate reduction	(Mannina et al., 2016)
Laboratory biological reactor	Fish canning wastewater treatment	Process inhibited for NaCl concentration>15 g/L; oil and greases increased wastewater biodegradability	(Cristóvão et al., 2016)
Sequencing batch reactor (SBR)	Study of different methods to characterize biodegradability and toxicity of hazardous pollutants	Diethyl phthalate was readily biodegradable, while acetylsalicylic acid was partly biodegradable, and all the other substances were refractory	(Tobajas et al., 2016)
SBR	Reduction of excess sludge production through para-nitrophenol addition	Negligible toxicity impact by nitrophenol on aerobic biomass	(Zuriaga-Agustí et al., 2016)
Various (review)	Effect of metal oxides nanoparticles on nitrification	Specific oxygen uptake rate could be used to estimate toxicity effects	(Kapoor et al., 2018)
Up-flow anaerobic sludge blanket (UASB)-MBR	Carbamazepine removal through anaerobic-aerobic treatment	Good biomass adaptation to carbamazepine; biomass activity decrease at high Sludge Retention Time (SRT)	(Moya-Llamas et al., 2021)
Laboratory	Analysis of the effects of	Antibiotics gave a major	(Menz et al.,

respirometer	18 pharmaceuticals on biomass composition by coupling respirometry and microbial community analysis	toxicity contribution, shaping microbial communities; overall toxicity calculated from single compound contribution	2017)

Table 2. Literature applications related to respirometry application for wastewater chemical oxygen demand (COD) fractionation and biomass characterization.

Investigated stream	Aim of the work	Main results	Reference
Sweeteners (aspartame, acesulfame K, sucralose)	Assessment of photochemical (ultra-violet + hydrogen peroxide) degradation efficiency	Non biodegradability of acesulfame K and sucralose; biodegradability of aspartame	(Jahani et al., 2020)
Pulp and paper wastewater	Evaluation of ozonation feasibility as tertiary wastewater treatment	Ozonation did not modify effluent biodegradability characteristics	(Mainardis et al., 2020)
Young landfill leachate	Comparison between municipal biomass and leachate-cultivated biomass	Higher biologic activity and heterotrophic active fraction in the leachate-cultivated sludge, with enhanced pollutant removal	(Corsino et al., 2020)
Landfill leachate	COD fractionation and kinetic analysis for	Obtainment of maximum heterotrophic growth yield,	(Insel et al., 2013)

	membrane bioreactor (MBR) treatment	half-saturation growth constant, maximum hydrolysis rate, hydrolysis half-saturation, endogenous decay rate	
Food additives	Determine the biodegradability of 20 food additives	9 compounds (including saccharine and aspartame) were highly biodegradable, 6 molecules were poorly degradable, and 5 substances were non-biodegradable	(Gatidou et al., 2020)
Slaughterhouse wastewater	Evaluation of the effectiveness of advanced oxidation process (AOP) (ultra-violet C, hydrogen peroxide, vacuum ultra-violet)	Reduction in treated effluent biodegradability after AOP	(Khaligh et al., 2017)
Pre-treated olive mill wastewater	Analysis of effluent biodegradability after chemical conditioning, ultrafiltration and nanofiltration	High effluent biodegradability was proved, highlighting the possibility of biological co-treatment with municipal sewage	(Naderi et al., 2017)
Winery wastewater	Assessment of wastewater biodegradability after	Low biodegradability increase; poor reduction of refractory compounds	(Moreira et al., 2015)

	biological and electrochemical treatment		
Tropical wastewater	Applying low Dissolved Oxygen (DO) nitrification to exploit soluble COD for denitrification	Influent characterization showed the predominance of particulate settleable (51%) COD fraction	(How et al., 2019)
Coke wastewater	Improvement of biomass understanding in combination with DNA sequencing	Reduced thiocyanate degradation in presence of ammonium, phenol and hydroxylamine	(Raper et al., 2019)
Landfill leachate and synthetic wastewater	Study nitrogen removal pathways in anammox processes in combination with microbial community analysis	Respirometry helped determine partial nitrification, anammox and denitrification activities	(Ke et al., 2015)
Municipal and industrial wastewater	Characterization of wastewater biodegradability	Strong variability in readily biodegradable COD (8-36%) and total biodegradable COD (48-75%)	(Hayet et al., 2016)
Particulate settleable solids from urban wastewater	Assessment of hydrolytic bacteria fraction	Small fraction of activated sludge efficient in performing hydrolysis	(Benneouala et al., 2017)

Municipal wastewater	Analysis of wastewater characteristics during wet weather events	Low load conditions limit respirometry applicability (insufficient sensitivity, uncontrolled oxygen request)	(Amerlinck et al., 2016)
Municipal wastewater	Evaluation of wastewater characterization methods during wet events	Limited respirometry applicability during wet weather; marked difference between respirometry and physicochemical methods	(Borzooei et al., 2021)
Municipal wastewater	Demonstrate respirometry applicability to constructed wetlands	High biodegradation potential; significant formation of storage products due to intermittent COD loads	(Ortigara et al., 2011)
Sanitary sewage	Determine heterotrophic and autotrophic kinetics in activated sludge systems with low aeration	Low DO operations were not limiting for simultaneous nitrification and denitrification	(Bueno et al., 2019)

Table 3. Literature outcomes related to respirometry application for wastewater treatment plant modelling.

Reactor	Scale	Aim of the work	Main respirometry outcomes	Reference

Conventional activated sludge (CAS), membrane bioreactor (MBR), sequencing batch reactor (SBR)	Laboratory and full-scale	Application of three respiration methods (based on exponential growth rate, maximum and endogenous respiration rate) to determine biomass kinetic parameters	The method based on modified maximum respiration rate was the best solution, due to its simplicity and accuracy	(Li et al., 2019)
Oxidation ditch	Full-scale	Assessment of optimum biomass solid retention time (SRT) to reduce operating costs	Determination of kinetic and stoichiometric parameters, showing that SRT increase could reduce the overall costs	(Muoio et al., 2019)
Biologic nitrogen removal (BNR) reactor	Full-scale	Plant mathematical simulation and calibration	Assessment of kinetic and stoichiometric parameters of aerobic and anoxic processes	(De Arana-Sarabia et al., 2018)
Aerobic Continuously Stirred Tank	Laboratory	Modelling palm oil effluent treatment through Activated	Chemical Oxygen Demand (COD) fractionation;	(Damayanti et al., 2010)

Reactor (CSTR)		Sludge Model No. 1 (ASM1)	determination of heterotrophic growth yield	
Oxidation ditch for low-temperature NH_3 removal	Full-scale	Mathematical model development	Determination of kinetic parameters to establish the best operating conditions for NH_3 removal	(Yang et al., 2013)
Three biological WWTPs (7,000 population equivalent, P.E., 18,200 P.E., 120,000 P.E.)	Full-scale	Plant simulation to support process upgrading	Satisfactory agreement between experimental and modelled data after calibration using respirometry outcomes	(Vitanza et al., 2016)
Biological WWTP (730,000 P.E.)	Full-scale	Full-scale wastewater treatment plant (WWTP) diagnosis	WWTP operated at overload conditions with low efficiency	(Arias-Navarro et al., 2019)
Anaerobic side-stream reactor (ASSR)	Full-scale	Evaluation of excess sludge reduction in comparison with CAS	Relevant reduction (-36%) in heterotrophic biomass activity and substrate consumption rate;	(Velho et al., 2019)

			sludge yield from 0.438 to 0.315 kg TSS/kg COD	
Ozonation reactor	Full-scale	Reduction of excess sludge production through ozonation	Significant decrease in heterotrophic growth yield (from 0.67 to 0.58 g cell COD/g wastewater COD)	(Gardoni et al., 2011)
Moving-bed MBR (MB-MBR) and MBR	Pilot-scale	Technology comparison	Gradual salinity increase allowed biomass adaptation, increasing process efficiency and stability	(Di Trapani et al., 2014)

Table 4. Literature studies related to photo-respirometry application for wastewater treatment.

Microalgae strains	Aim of the work	Main outcomes	Reference
Chlorella and *Scenedemus* sp.	Testing a novel photo-respirometric system to determine oxygen production rate (OPR) and oxygen uptake rate (OUR)	Photo-respirometry could be employed in monitoring procedures of algal ponds/photobioreactors	(Rossi et al., 2018)
Chlorella and	Evaluate operating	Definition of a modelling tool	(Rossi et

Scenedemus sp.	conditions effects on a microalgae-bacteria consortium	to predict algae growth rate as a function of environmental conditions (temperature, pH, irradiance, dissolved oxygen)	al., 2020b)
Chlorella and *Scenedemus* sp. (green microalgae), *Synechococcus* and *Synechocystis* spp. (cyanobacteria)	Evaluate free ammonia effects on photosynthesis	Cyanobacteria more prone to inhibition by free ammonia than green microalgae; mixed consortia more resistant than monocultures	(Rossi et al., 2020c)
Scenedesmus almeriensis	Study a new photo-respirometry method to assess the main microalgae metabolic processes	Definition of a protocol (including dark and light periods), measuring OPR in presence of different substrates	(Sánchez-Zurano et al., 2020)
Chlorella sp.	Estimate kinetic and stoichiometric parameters for endogenous respiration, nitrification and chemical oxygen demand (COD) oxidation	Possibility to use photo-respirometry to estimate OPR and OUR, allowing to improve photo-bioreactors design	(Petrini et al., 2020)
Chlorella	Study microalgae	Similar half-saturation	(Pastore et

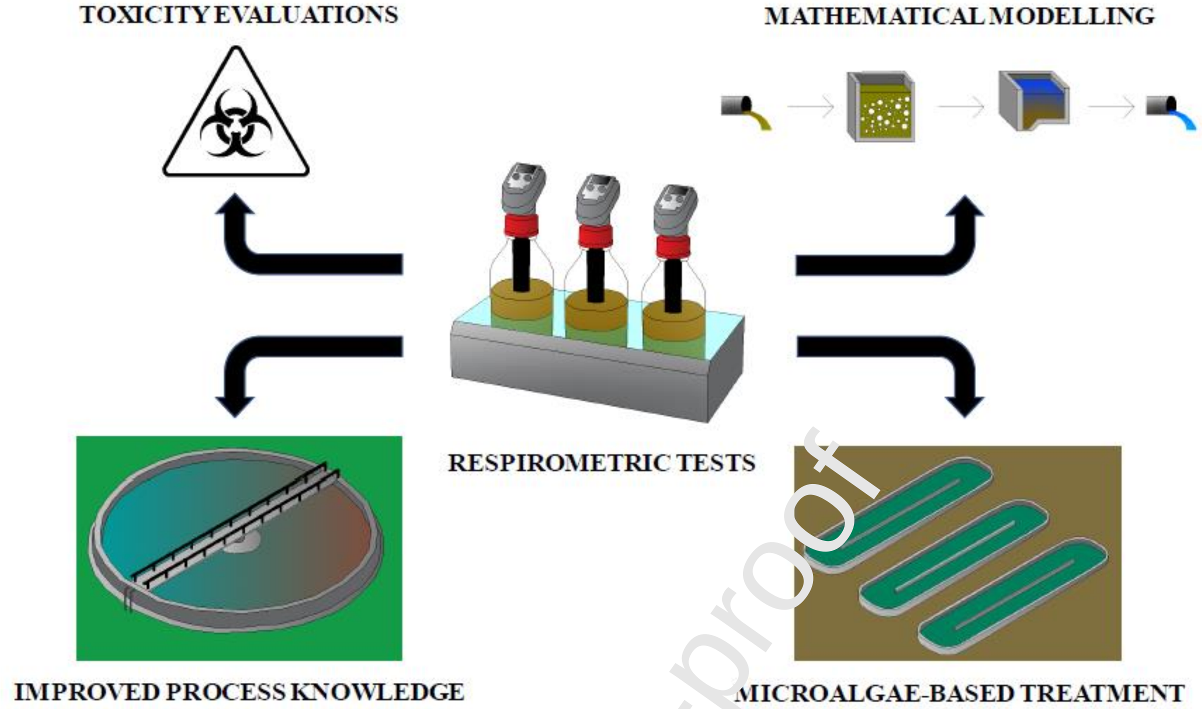
protothecoides	kinetics related to nitrogen consumption in batch and continuous mode	constants for different nitrogen species; algae prevalence for ammonium rather than nitrate	al., 2020)
Synechocystis sp.	Assessment of light and CO_2 effects on process regulation	High COD (68%) and nutrient (P=96%, N=66%) removal for high-strength wastewater (COD=6,000 mg/L, N=600 mg/L, P=90 mg/L) treatment	(Trentin et al., 2019)
Chlorella protothecoides	Understand the possibility to exploit the oxygen produced by photosynthesis to support activated sludge growth	Possibility to quantify oxygen reduction in presence of biodegradable COD in co-cultivation with activated sludge bacteria	(Sforza et al., 2018)

Declaration of competing interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Graphical abstract



Highlights

- Respirometry tests are useful for COD fractionation and model calibration
- Short- and long-term toxicity and inhibitory effects can be evaluated as well
- Innovative approaches include microalgae, fungi and MBBR characterization
- Criticisms are diluted effluents analysis and industrial streams characterization
- The coupling with microbial community analysis can give further process insights