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## A COMMENTARY ON THE RESPIROMETRIC EVALUATION OF BIODEGRADABLE COD FRACTIONS IN INDUSTRIAL WASTEWATER

### UWAGI O RESPIROMETRYCZNYM WYZNACZANIU BIODEGRADOWALNYCH FRAKCJI CHZT W ŚCIEKACH PRZEMYSŁOWYCH

#### Abstract

This paper presents considerations on selected methodological aspects of respirometric COD fractionation in industrial wastewater which may differ from more common applications of such methodology for municipal wastewater. This concerns: the presence of interfering substances; problems with the elimination of nitrification; the importance of recognising biomass metabolism; differences of growth coefficient; the inclusion of non-canonical COD fractions and toxic effects. The discussion is based on a literature review and the authors' own experience. As a conclusion, a preparatory procedure is proposed to assure the correct performance of respirometric COD fractionation for industrial wastewater, especially for more complex chemical effluents.

**Keywords:** COD fractionation, industrial wastewater, respirometry

#### Streszczenie

Artykuł przedstawia rozważania na temat wybranych aspektów metodycznych respirometrycznego wyznaczenia frakcji ChZT w ściekach przemysłowych, które mogą różnić się od bardziej rozpowszechnionych zastosowań tej metody dla ścieków miejskich. Obejmuje to obecność substancji przeszkadzających, problemy z eliminacją nityfikacji, rozpoznanie metabolizmu biomasy, zróżnicowanie współczynnika wzrostu, uwzględnianie niekanonicznych frakcji ChZT i efektów toksycznych. Dyskusję oparto na przeglądzie literatury oraz własnych doświadczeniach autorów. W podsumowaniu zaproponowano przygotowaną procedurę mającą zapewnić prawidłowe wykonanie respirometrycznego wyznaczenia frakcji ChZT dla ścieków przemysłowych, zwłaszcza tych o bardziej skomplikowanym, „chemicznym” charakterze.

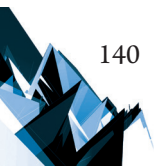
**Słowa kluczowe:** frakcjonowanie ChZT, respirometria, ścieki przemysłowe

## 1. Introduction

Traditional methods for assessing the organic contamination of wastewater such as total chemical oxygen demand (COD) or biochemical oxygen demand (BOD) measurements are not sufficient for the proper control of modern biological wastewater treatment plants (WWTPs). The advanced biological nutrients removal and the mathematical modelling of biological treatment processes require a more detailed characterisation of organic substrates. This issue is pursued as a so-called COD fractionation; this is the division of all wastewater organic contamination (expressed as COD) into fractions on the basis of their treatability and other parameters. In case of biodegradable industrial effluent, such COD fractionation is even more important than for municipal wastewater as it could help to evaluate the potential effectiveness of the removal of organic contaminants and provide other important information.

COD fractionation of municipal wastewater can be accomplished by several methods, fully described in previous literature [3, 14]. In Poland, guidelines of German ATV-131P are fairly frequently applied; these rely upon filtered and unfiltered COD measurements of influent and effluent wastewater as the basis for COD division into fractions. Such a simplified approach, and possibly other methods employing physical-chemical separation (e.g. filtration/coagulation), may work reasonably effectively for municipal effluents, which are of more or less similar characteristics. However, industrial wastewater, especially from the chemical industry, is more complex. Therefore, use of respirometric methods of COD fractionation seems more appropriate. In these methods, electron acceptor (oxygen or nitrate, depending on conditions) consumption by biomass is measured and then recalculated into the COD of the degraded substrate. Their advantage is that much more information can be gained from a test regarding the characteristics of wastewater organic contamination, specifically: biodegradability and toxicity (both of particular importance for industrial effluents), and degradation kinetics.

There are several approaches for performing respirometric tests; these have been fully discussed in literature [22]. The most reliable seem to be those which are based on the 'liquid phase, static gas' principle, with the liquid phase either static or flowing, as this enables eliminating the need for the inclusion of oxygen mass transfer (from gas to liquid phase) in calculations. Automatic instruments which can operate under such conditions are available commercially, but it is also possible to perform such a test using the following simple equipment: a closed measuring chamber (e.g. a bottle) with a dissolved oxygen (DO) sensor and mixing of the content, periodically re-filled with mixed liquor from a large continuously aerated tank in which the wastewater of interest is dosed at the start of measurements. All vessels should be placed in a water bath, maintaining a constant mixed liquor temperature, typically 20 or 25°C. The decrease of DO concentration in the measuring chamber is recalculated into the oxygen uptake rate (OUR,  $\text{mgO}_2/\text{min}\cdot\text{dm}^3$ ), and the OUR changes over time create a respirogram (sometimes termed 'a short-term BOD'). Evaluation of such an OUR profile enables the identification of degradable COD fractions and their quantification. In a simpler approach, this evaluation could be achieved by delineating areas under the



OUR curve which correspond to oxygen utilisation for the degradation of individual COD fractions, using characteristic points [3, 15]. The advanced method involves the application of simulation models such as International Water Association activated sludge models (ASMs). Non-biodegradable fractions can be estimated by assessing a biodegradable COD in a respirometric test and subtracting it from the total COD; this is done using filtered and unfiltered wastewater for evaluation of soluble and particulate inert fraction respectively [20].

Respirometric COD fractionation has long been applied for the analysis of municipal wastewater. However, such analyses with industrial discharges are sometimes very specific, and the methodology commonly used for municipal wastewater cannot be transferred directly and without thorough consideration, as that may lead to serious mistakes. In this paper, the most important methodological aspects (according to authors' experience) are addressed. These are discussed in the context of the literature review and also illustrated by examples from the authors' own research.

## 2. General remarks

The use of biomass well-adapted to tested wastewater is of even more importance than in the case of municipal effluents. Several chemicals are not degraded by non-adapted activated sludge; furthermore, the presence of toxic substances requires the prior acclimatisation of biomass. Industrial wastewater is often nutrient-deficient; thus, the supplementation of nitrogen, phosphorus and micronutrients may be necessary. Suggestions of trace element solutions and their dosage can be found in literature [26]. Another important issue to ensure appropriate conditions for respirometric testing is pH regulation to within the optimal range for used biomass.

## 3. Interfering substances

Hydrogen peroxide ( $H_2O_2$ ), present in high concentration in effluents from wood pulp and textile bleaching, and in wastewater pre-treated with this oxidant (e.g. pre-oxidation by the Fenton process), may cause problems in respirometric COD fractionation. This may concern: 1) overestimation of the total COD in analytical determination when using the dichromate method; 2) dissolved oxygen supersaturation, which could make the OUR measurements unreliable; 3) possible disturbance of biomass activity. One of the methods proposed for avoiding  $H_2O_2$  interference on analytical COD measurement is  $H_2O_2$  reduction with sodium sulfite ( $Na_2SO_3$ ; [28]). Removal of the remaining peroxide with  $Na_2SO_3$  was tested by the authors for chemo-thermo-mechanical pulping effluent from the paper industry. In such a way, a correct respirogram was obtained (Fig. 1). However, attention must be given to eliminate residual excess of  $Na_2SO_3$ ; otherwise it would cause additional oxygen demand during measurements.

Similar problems can be expected with the presence of chlorine in wastewater, disturbing DO measurements. Inorganic compounds readily oxidised by dissolved oxygen, such as sulfite and

sulfide, should also be removed before the determination of organic COD fractions. The presence of nitrite requires the use of a nitrataion (the second step of nitrification) inhibitor (see below).

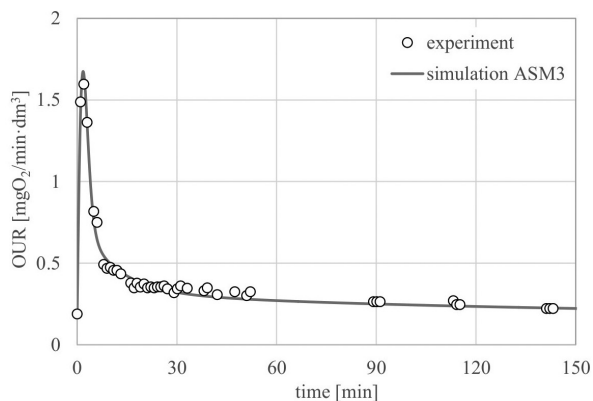


Fig. 1. Batch respirometric test with chemo-thermo-mechanical pulping wastewater after hydrogen peroxide removal: experimental results and simulation using ASM3-type model

#### 4. Inhibition of nitrification

In aerobic conditions, oxygen can be consumed not only by heterotrophic bacteria but also by nitrifying organisms present in biomass used for respiration testing. Therefore, the respirometric determination of COD fractions is possible only by fitting an elaborate simulation model covering both the oxidation of organic matter and the oxidation of nitrogen compounds to the experimental results. Such an approach is problematic as a large number of variables and coefficients may result in great estimation inaccuracy. Thus, the most common practice while performing respiration tests is to suppress nitrification using nitrification inhibitor allylthiourea (ATU) at a dose of 10–20 mg/dm<sup>3</sup>. In this way, only oxygen uptake for organic compounds degradation is to be considered.

ATU is a strong selective inhibitor of the ammonia monooxygenase enzyme, stopping the first stage of nitrification – the oxidation of ammonia to nitrite (nitrification [9]). However, it has recently been reported that the adaption of biomass to this chemical may occur, resulting in incomplete inhibition of nitrification or even ATU degradation [17, 22]. It seems likely that such adaptation can be found especially in the case of biomass from WWTPs treating industrial effluents [18]. ATU may not be effective against ammonia-oxidising archaea [16], which could also occur in activated sludge.

An alternative nitrification inhibitor occasionally used in respiration tests is nitrapyrin (2-chloro-6-(trichloromethyl)pyridine) [22, 24]. It is also recommended for use in 5-day BOD tests (e.g. Hach nitrification inhibitor formula 2533, containing 2% nitrapyrin coated on sodium sulfate). However, nitrapyrin has low water solubility (40 mg/dm<sup>3</sup>) which makes its use questionable when high or rapid dosing is required. Other alternatives to ATU can be selected from several other compounds exhibiting selective inhibiting properties towards

nitrification and used as additives to fertilizers. Examples of such substances can be found in literature [19, 30]. The efficiency of selected nitrification inhibitors should be verified by comparing biomass respiration rates before and after subsequent pulse additions of ammonia and an inhibitor (an example can be found in [9]).

In the case of nitrite presence in wastewater, the use of a selective inhibitor of first step nitrification, such as ATU, will not eliminate oxygen uptake by the oxidation of nitrogen compounds. The use of a nitrification (oxidation of nitrite to nitrate) inhibitor is then necessary. Chlorate is commonly used for this purpose at a concentration of 10–20 mM [18, 23]. However, it should be noted that this compound is not always effective [18]. Another selective inhibitor of nitrite oxidation is azide, effective in doses of 0.24  $\mu\text{M}$  [9].

## 5. Type of metabolism

Older biodegradation models (ASM1 and similar) assumed that the substrate present in wastewater was directly utilised for biomass growth. In newer models (e.g. ASM3), a different approach for substrate usage is adopted, assuming that substrate is first taken up and stored, then growth occurs at the expense of stored products. This type of metabolism is supposed to develop in biomass subjected to alternate ‘feast and famine’ phases (availability and absence of external substrate). Such conditions may occur, for example, in plants with anoxic and aerobic zones/periods with variable loading, while stable loading and aeration only may prefer ASM1 – type behaviour of biomass. More recent investigations (e.g. [10]) indicate that in reality, the utilisation of wastewater substrate by biomass is even more complex – a part is directly utilised for growth, while the other part is stored. A simulation model of such simultaneous metabolism can be found, for example, in a paper by Fan et al. 2012 [8].

Although modelling of full-scale WWTPs can be successfully achieved using ASM1 only [25], conditions of batch scale respirometric tests require the selection of the correct metabolic model to avoid considerable estimation errors. For example, the application of traditional respirometric methods of readily degradable COD fraction determination, either at a low food to microorganism ratio (F/M) [7] or at a high F/M [29] with biomass exhibiting substantial substrate storage behaviour will produce a serious underestimation of the actual concentration of this fraction. The identification of an appropriate substrate utilisation mechanism could be accomplished by running a batch respiration test with the biomass of interest and an easily biodegradable soluble reference substrate, such as sodium acetate. The existence of substrate storage can be recognised by a characteristic ‘tailing’ of the initial peak – the OUR does not drop to the endogenous value but remains above it, gradually declining (Fig. 3). By contrast, direct utilisation of substrate manifests in one-phase respirogram, where the OUR reaches an initial peak and then quickly returns to the endogenous respiration value with no apparent ‘tail’.

## 6. Growth coefficient

An issue of critical importance for proper respirometric determination of COD fractions is the correct adjustment of the heterotrophic biomass yield (growth) coefficient  $Y_H$  (mg COD biomass/mg COD degraded). This factor relates the measured amount of oxygen  $\Delta O_2$  utilised in respirometric tests to the amount of degraded COD ( $COD_{degr}$ ), according to the equation [22]:

$$COD_{degr} = \frac{\Delta O_2}{(1 - Y_H)} \quad (1)$$

Thus, even a small inaccuracy of the assumed  $Y_H$  value may result in a serious error of COD estimation. For example, replacement of the actual value of 0.60 by 0.67 (which is the default value in the ASM1 model for domestic wastewater) will cause a 21% overestimation of biodegradable COD fractions.

Considering domestic wastewater from different settlements,  $Y_H$  remains fairly constant. Orhon et al. [20] quoted experimentally determined values in the range of 0.62–0.67, which is close to the default values of ASM1 (0.67) and ASM2 (0.63). However, in the case of industrial effluents,  $Y_H$  is much more variable. The range of experimental values reported by Orhon et al. [20] is 0.55–0.69. The authors' own experiments with specific chemical industry wastewater indicated a value of ~0.58.

All the abovementioned evidence points to the necessity for the experimental determination of  $Y_H$ , especially in the case of more complex wastewater. A typical procedure of such estimation involves running a respiration test with the addition of the wastewater of interest, and with periodical measurements of soluble COD. A removal of soluble COD is compared to corresponding total oxygen consumption reduced by oxygen utilized for endogenous respiration by biomass at the same time (example of such determination could be found e.g. at [24]).  $Y_H$  can then be calculated through transformation of Equation (1). However, attention must be paid to two issues. Firstly, the added wastewater of interest has to be filtered/coagulated etc. so that it contains only soluble substrates; otherwise, oxygen consumption would also include the degradation of particulate substrate, which would be not accounted for in soluble COD measurements and would result in the underestimation of  $Y_H$ . Secondly, in the case of substrate storage metabolism (such as in ASM3), instead of the direct assimilation of readily biodegradable substrate (such as in ASM1), the removal of soluble COD precedes its utilisation for growth. The automatic application of the abovementioned procedure of  $Y_H$  determination would then lead to overestimation of its value.

If biomass exhibit considerable substrate storage behaviour, a method proposed by Karahan-Gül et al. [15] for the determination of the storage yield of  $Y_{STO}$  [mg COD substrate stored/mg COD substrate removed] should be applied. With this method, oxygen utilisation corresponding to the area of initial respirogram peak limited by a line linking the starting endogenous respiration point with the turning point between peak's descending slope and its 'tail' is compared to a soluble COD decrease, using transformed Equation (1). Next, the yield coefficient for growth on the stored substrate  $Y_H$  can be derived from simulation of



a whole test. Alternatively, this coefficient could be roughly estimated with results from a long respirometric test with low F/M conditions, continued until the OUR is close to endogenous respiration, using the Equation (2):

$$Y_H = \frac{1 - \frac{\Delta O_{2,tot}}{COD_{degr,tot}}}{Y_{STO}} \quad (2)$$

where  $\Delta O_{2,tot}$  is the total oxygen consumption reduced by oxygen utilization for endogenous respiration and  $COD_{degr,tot}$  is the total soluble COD removal. This relationship is simplified as it does not include oxygen utilisation for the oxidation of the stored substrate ('respiration' of storage products in ASM3). It should be noted, however, that this respiration in a batch test with a low F/M ratio is low compared to the oxygen uptake for storage and growth; thus, the estimation error of  $Y_H$  is small.

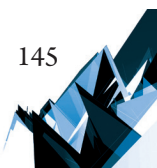
## 7. Non-canonical COD fractionation

With regard to municipal wastewater, four COD fractions are usually distinguished: soluble easily biodegradable  $S_s$ ; soluble inert (non-degraded)  $S_i$ ; particulate slowly biodegradable (hydrolysable)  $X_s$ ; and particulate inert  $X_i$ . Together, these comprise the total COD of wastewater, which should be equal to COD estimated by a chemical method. In the case of industrial effluents, such division may not be sufficient to describe the degradation kinetics of organic substrates; this requires the accommodation of additional fractions. Their biodegradation may proceed simultaneously (as in ASM models) or sequentially, i.e. degradation of the second fraction takes place after the first fraction is exhausted. The modelling of the latter case can be achieved through the inclusion of a 'switch' function in the degradation/growth rate equation for the second fraction (e.g. in [6]):

$$\frac{K_{S1}}{K_{S1} + S_{S1}} \quad (3)$$

where  $K_{S1}$  is a saturation constant for the first fraction and  $S_{S1}$  is the first fraction concentration. Such a situation may take place when the generation and accumulation of an intermediate product occur with its subsequent utilisation for growth [13]. Babu and Varghese [2] reported that in the mixture of cyclohexanone and cyclohexanol, degradation of the second compounds only started when about 90% of cyclohexanone had been exhausted, while both chemicals were degraded without delay when added separately.

Cokgor et al. [6] described a case of chemical wastewater (mainly from personal care and pharmaceutical production) with three separate sequentially used readily biodegradable fractions and an additional slowly biodegradable (hydrolysable) fraction. Helle and Duff [11] identified two to five readily biodegradable fractions of different biodegradation rates in bleached Kraft mill (wood pulping) effluent, all of which were degraded simultaneously. Similar conclusions were drawn by the author for another stream of paper industry



wastewater [4]. Industrial wastewater composed of three readily biodegradable fractions, all of them simultaneously degraded, was also described by Coen et al. [5]. Orhon et al. [20] also recommended adopting a dual hydrolysis model for industrial wastewater with high concentration of slowly biodegradable substrate, assuming the existence of a rapidly hydrolysable fraction (soluble) and slowly hydrolysable fraction (particulate).

The initial recognition of COD fractionation in wastewater can be achieved by analysing a batch respirogram curve. Peaks or plateaus followed by rapid drops of the OUR indicate the presence of readily degradable fractions; gradual declines denote hydrolysable substrates. A more precise choice of COD fractions division could be accomplished by the fitting of a customised model to experimental data using specialised simulation programs, e.g. AQUASIM [1]; this is free software allowing users to develop their own models and calibrate them on the basis of experimental data. Typically, such models are simplified to cover only activity of heterotrophic organisms: their growth, endogenous respiration, storage (if needed) and hydrolysis of substrate. Figure 2 presents an example of a respirogram obtained for paper industry wastewater. Its shape suggests the existence of at least two readily biodegradable fractions, used simultaneously, and at least one rapidly hydrolysable fraction. This was simulated using ASM3-type model, assuming substrate storage.

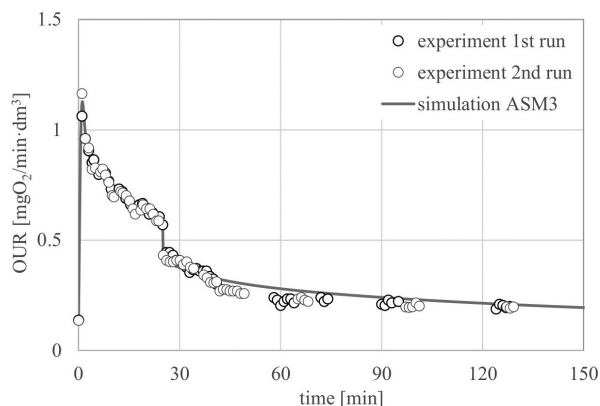


Fig. 2. Batch respirometric test with paper industry wastewater stream: experimental results (two runs) and simulation using an ASM3-type model with two readily degradable fractions

## 8. Toxic effects

An additional quality of respirometric measurements is the possibility for the assessment of inhibitory or toxic effects of substances that could be present in industrial wastewater. Traditionally, for non-biodegradable toxicants, such test is done by comparison of maximal exogenous (after deduction of endogenous respiration) OUR obtained with an easily degradable reference substrate (e.g. acetate) dosed together with different concentrations of tested chemical. However, the factor affecting toxic response can be not only the size of the toxicant dose but also the time of contact between the biomass and the tested compound.



Spanjers and Vanrolleghem [22] reported three types of contact time-dependent behaviour: a constant effect; an increasing (stronger) effect; a decreasing effect (adaptation of biomass). According to these authors, sometimes even a stimulation could be noted, that is, OUR can be higher with toxicant than without it. Running such a toxicity test with a mixed model substrate containing both COD and ammonia nitrogen may allow the simultaneous determination of toxic effects against heterotrophic and nitrifying microorganisms.

More information about toxic effects on different processes of biomass metabolisms can be obtained through the evaluation of a whole OUR profile of a respiration test and fitting an appropriate simulation model [12]. This also refers to biodegradable toxicants, where a self-inhibition of biodegradation (substrate inhibition) may occur. This manifests in a slow exponential increase of OUR in an initial phase of a test. A typical example of such a substance is phenol. Commonly, such a phenomenon is simulated using Haldane-type biodegradation kinetics; examples of such applications can be found in [6] and [21].

An initial gradual increase of OUR can also be observed in tests without toxic substances. In such cases, it should be ascribed to transient phenomena associated with sudden changes of substrate concentration, such as the activation of enzymes, substrate diffusion into biomass flocs, dynamics of dissolved oxygen measurements, etc. [27]. These authors [27] proposed the following formula to include this response in simulation models:

$$\mu_{obs} = \left(1 - e^{-\frac{t}{\tau}}\right) \cdot \mu \quad (4)$$

where  $\mu$  is the maximum growth rate of microorganisms in given conditions,  $\mu_{obs}$  is the actual (observed) growth rate,  $t$  is time and  $\tau$  is the time constant. Figure 3 presents a respirogram obtained for a caprolactam (cyclic amide of 6-aminohexanoic acid) degradation test with an attempt to fit a ASM3-like degradation model, including both Haldane degradation inhibition kinetics and the transient response formula, which gave the closest fit compared to the individual application of these equations.

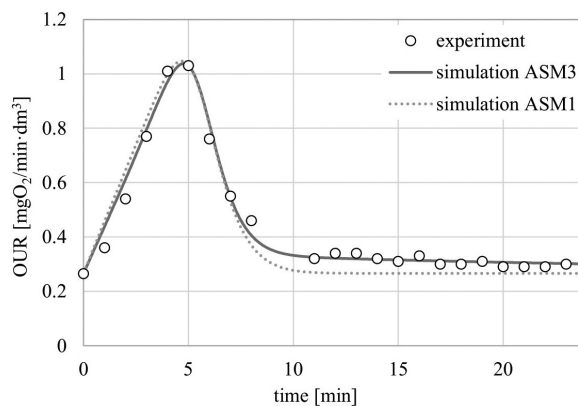


Fig. 3. Batch respirometric test with caprolactam: experimental results and ASM3- and ASM1-type simulations including Haldane inhibition kinetics and transient response formula

## 9. Conclusions

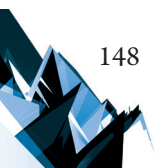
On the basis of the above considerations, a preliminary procedure can be proposed as a prerequisite for the correct evaluation of COD fractions in industrial wastewater. This procedure is as follows:

- ▶ used biomass must be well-adapted for investigated chemicals/wastewater streams;
- ▶ wastewater should be checked with regard to potentially interfering substances such as oxidising and reducing agents (e.g. peroxide, chloride, sulfite, sulfide) and nitrite;
- ▶ the effectiveness of nitrification inhibitors should be verified for used biomass; if nitrite is present in the tested wastewater inhibitors of nitrification should be checked as well;
- ▶ the type of metabolism should be identified for used biomass, using a reference substrate (e.g. acetate): if substrate is utilised directly (as in ASM1 model) or if it is stored (as assumed in ASM3);
- ▶ the growth coefficient(s) should be estimated for the chemicals/wastewater of interest.

After applying the above procedure, proper tests can be run, maintaining optimum pH and providing adequate nutrients and micronutrients. COD fractions identification and quantification should preferably be performed by fitting simulation models. This, however, should be done in a flexible manner – it might be necessary to customise the model by changing the canonical form of ASMs in terms of COD fractions division and/or biodegradation kinetics.

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