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Quality improvement in determination of chemical oxygen demand in samples considered difficult to analyze, through participation in proficiency-testing schemes

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Chemical oxygen demand (COD) is a critical analytical parameter in waste and wastewater treatment, more specifically in anaerobic digestion, although little is known about the quality of measuring COD of anaerobic digestion samples. Proficiency testing (PT) is a powerful tool that can be used to test the performance achievable in the participants' laboratories, so we carried out a second PT of COD determination in samples considered "difficult" to analyze (i.e. solid samples and liquid samples with high concentrations of suspended solids). The results obtained (based on acceptable z-score values) may be considered satisfactory. When compared with the results of a previous similar scheme, the overall performance improved by around 30%, again demonstrating that analytical performance can be improved by regular participation in PT.
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Keywords: Anaerobic digestion; Chemical oxygen demand (COD); Interlaboratory study; Liquid sample; Proficiency testing (PT); Solid sample; Suspended solids; Waste treatment; Wastewater treatment

1. Introduction

The performance and the control of anaerobic processes are generally assessed by monitoring different analytical parameters, including chemical oxygen demand (COD). These systems have an organic-matter content supplied by water and suspended solids from waste and biota. However, hardly anything is known about the quality of COD measurements from anaerobic-reactor samples. From a scientific point of view, it is essential to ensure that the data produced are of sufficient trueness and precision to serve as a basis for drawing meaningful conclusions about the performance of reactors and the comparative study among different laboratories.

This contribution is the third research report that deals with the analytical determination of COD using both solid and liquid samples with high concentrations of suspended solids. The first contribution looked at the proposition of a modified analytical method for COD determination [1], whereas the second focused on the first COD proficiency testing (PT) of the anaerobic digestion groups (1st COD-PT^{ADG}), compiling data from laboratories mainly specializing in anaerobic digestion [2].

The results obtained were unsatisfactory because the majority of the participating laboratories obtained inappropriate performances. This showed the difficulties that lie in determining COD in these types of sample. However the results were not surprising, because laboratories unacquainted with PT schemes invariably fail to produce satisfactory results.

There are several reasons for participating in a PT scheme:

- evaluation of the performance and continuous monitoring;
- evidence of reliable results;
- identification of problems related to the systematic nature of assays;
- the possibility of taking corrective and/or preventive measures;
- evaluation of the efficiency of internal controls;
- determination of the performance characteristics and validation of methods and technologies;
- standardization of the activities in the market; and,
- national and international recognition of assay results [3].

Despite the fact that a single result in a PT scheme simply reflects the quality of the performance of a laboratory at any given point in time and that the extrapolation from success in a PT scheme in everyday analytical work is an assumption, frequent participation in PT schemes is highly recommended and can help provide insights into the level of quality within a laboratory. Moreover, observing that another laboratory finds approximately the same measurement result from the same measurands provides analysts with great comfort and gives them self-confidence – confirmation always gives a nice feeling.

PT schemes are therefore welcome because they provide a clear, straightforward way of evaluating the accuracy (trueness and precision) of results obtained by different laboratories. The participation in PT is also considered a powerful tool for detecting and removing sources of common errors due to the lack of quality control (QC) within a laboratory.

The 2nd COD-PT^{ADG} was organized with the aim of comparing the data from both the 1st and 2nd COD-PTs and of determining if PT schemes improve the performance of participant laboratories.

2. Organization of the PT scheme

This study is the second attempt at a worldwide interlaboratory comparison of analytical COD determination using solid samples and liquid samples with high concentrations of suspended solids. These samples are considered to be difficult to analyze and are problematic in the corresponding determinations. The scheme was organized by the "Reuse of Wastes and Wastewater Treatment Group", of the Instituto de la Grasa (IG) of the Spanish National Research Council (CSIC).

The PT coordinator and collaborators were responsible for:

- designing the overall scheme;
- preparation, testing and distribution of selected samples;
- distribution of instructions among the participating laboratories;
- collection of data, their statistical treatment and feedback of results to participants.

This PT was carried out according to the International Harmonized Protocol for the PT of Analytical Chemistry Laboratories [4].

The PT coordinator sent invitations to participate in the 2nd COD-PT^{ADG} in June 2009. The test took place between 15 September and 15 October 2009. Each participating laboratory received four samples, together with technical guidelines on how to proceed with the measurements. A total of 20 laboratories from 13 countries agreed to participate. All the participating laboratories were highly motivated about taking part in the PT scheme, as the full return rate of data proved. All participating laboratories provided feedback, first about their own performance, and second about the general performance, all of which was reported anonymously.

3. Materials and methods

3.1. Materials

3.1.1. Description of samples. To carry out the 2nd COD-PT^{ADG}, four different samples were selected. These samples were divided into two main groups: solid samples (SS) and liquid samples with a high suspended solid concentration (LS-HSSC):

- Sample 1 (SS 1). Gelatin (Gel). Pure powder protein used as a solidifying agent in the preparation of microbiological culture media to identify proteolytic microorganisms (gelatinase producers). The gelatin used was supplied by Panreac-Spain (Code 403902).
- Sample 2 (SS 2). Sewage sludge (SewS). A sewage sludge produced by Resource Technology Corporation (USA and UK) and provided for characterization as a new certified reference material (including 19 metals as well as COD, Kjeldahl nitrogen and total phosphorus).
- Sample 3 (LS-HSSC 1). Sunflower-oil cake (SuOC). A by-product made up of the part of whole sunflower seeds that remains after oil-extraction processes. It is a heterogeneous substrate that can be broken down into three main components: a proteinaceous fraction, a lignocellulosic fraction and a soluble fraction. The sample was prepared with 5 g of raw material.
- Sample 4 (LS-HSSC 2). Mung bean (MB). The seed of *Vigna radiata*, which is native of Asia (Bangladesh, India and Pakistan). This seed is also known as green bean, green soya, and green gram. Its beans are small, ovoid in shape, green when raw and yellow when dehusked. The sample was also prepared with 5 g of raw material.

3.1.2. Preparation of samples. The suitability and the quality of the test materials distributed are fundamental for the effectiveness of a PT scheme. The two main criteria for suitable test material are that:

- it resembles, as closely as possible, the real samples with which a laboratory routinely works; and,
- variations in the composition of the samples of the test material distributed to participants are kept to the minimum [5].

The PT material was prepared by the PT coordinator. Although his working laboratory has not implemented a quality system accredited according to ISO 17025, he is very experienced in this field and has been involved in different laboratory QC systems, so all the characteristics that could affect the integrity of the test were taken into consideration, including the homogeneity and the stability of the samples.

Considering that different particle-size fractions of the solid samples dispatched would lead to a lack of homogeneity with respect to COD determination, a control of particle size was carried out by sieving the substrates selected to the desired size.

Taking into account that the moisture content of solid-substrate samples can vary with ambient humidity, the participants were requested to report results on a dry-weight basis.

Samples 3 and 4 were two liquid samples with high concentrations of suspended solids that had to be reconstituted in-laboratory by adding 200 mL of distilled water to the spiked amount of solid content weighed into the containers. All participants were instructed to stir the samples for 1 h before COD analysis and during the sampling procedure.

3.1.3. Characterization of samples. All samples distributed were analyzed in the laboratory of the PT coordinator. Three replicates of different parameters (moisture, organic content and elemental composition) were prepared for each sample. Table 1 summarizes the main characteristics of the samples selected.

3.1.4. Homogeneity of samples. Immediately after packaging the samples, they were tested for sufficient homogeneity using the standard analytical method developed in the laboratory of the PT coordinator and used on a routine basis. To check for sufficient homogeneity, the protocol devised by Fearn and Thompson [6] was used. In accordance with their approach, three tests were carried out to estimate the corresponding experimental statistical parameters and compared with their theoretical critical values:

Table 1. Characterization of the solid samples used in the 2nd COD-PT^{ADG}

	Sample 1 *(Gel)*	Sample 2 *(SewS)*	Sample 3 *(SuOC)*	Sample 4 *(MB)*
Particle size (mm)	N.D.[a]	0.2–1	0.125–0.355	0.125–0.500
Moisture (%)	8.0 ± 0.3	10.0 ± 0.4	8.0 ± 0.4	9.0 ± 0.3
Organic content (%TS)	100.0 ± 0.1	60.3 ± 0.5	93.0 ± 0.5	97.0 ± 0.5
Chemical Composition (%-VS)				
Carbohydrates	–	N.D	55.5	72.4
Fat	–	N.D	1.1 ± 0.2	1.5 ± 0.2
Protein[b]	100	N.D	26.4 ± 0.6	23.1 ± 0.6
NDF	–	N.D	40 ± 1	5.0 ± 0.5
Elemental Analysis (%-TS)				
C	48.2 ± 0.3	32.9 ± 0.5	45.9 ± 0.6	44.6 ± 0.6
H	6.5 ± 0.3	4.5 ± 0.1	6.3 ± 0.1	6.8 ± 0.3
N	18.4 ± 0.1	4.8 ± 0.1	5.4 ± 0.4	4.4 ± 0.1
S	0.6 ± 0.1	1.4 ± 0.1	0.20 ± 0.04	0.07 ± 0.01
O	26.2 ± 0.4	16.7 ± 0.7	35.2 ± 0.8	41.1 ± 0.6
Theoretical Oxygen Demand (*ThOD*-mg $O_2 \cdot g^{-1}$ TS)	1236	956	1249	1240

[a]N.D.: Not determined.
[b]%NTK x Nitrogen-to-protein conversion factor (5.5).

Table 2. Summary of analytical procedures utilized by participating laboratories in 2nd COD-PT^{ADG}

Lab[1]	Method[2]	Digestion Reagent			Acid Reagent[3]		$HgSO_4$	Water	End Point[4]
		$K_2Cr_2O_7$			H_2SO_4-$AgSO_4$				
		Vol. (mL)	Conc. (N)	Vol. (mL)	Conc.[c] (%)	Conc.[d] (g/L)		Vol. (mL)	
1[a]	(2) OR-HCM	25	1.0	20	98	10	Yes	0	TT [g]
1[b]	(1) OR-LCM	5	0.241	15	98	10	Yes	10	TT
2	(4) CR-SM				99	10	No	No	SP[e]
3	(1) OR-LCM	20	0.5	30	98	5	Yes	10	TT
4[a]	(5) CR-KSM								SP
4[b]	(5) CR-KSM								SP
5	(2) OR-HCM	10	1.0	30	98	10	Yes	10	TT
6[a]	(2) OR-HCM	15	1.0	45	98	9.4	Yes	20	TT
6[b]	(3) CR-TM	1.5	0.21	3.5	98	10.7	Yes	0	TT
7	(2) OR-HCM	10	1.2	30	98	10	Yes	0	TT
8	(4) CR-SM	1.5	0.2148	3.5	98	10	Yes	2,5	SP
9	(2) OR-HCM	20	1.2	25	98	10	Yes	10	PT[f]
10	(2) OR-HCM	20	1.2	30	98	10	Yes	10	PT
11	(1) OR-LCM	50	0.25	50	98	10	Yes	25	TT
12	(5) CR-KSM								SP
13	(2) OR-HCM	20	1.0	30	98	10	Yes	20	TT
14	(1) OR-LCM	25	0.25	75	96	10.6	Yes	0	TT
15	(2) OR-HCM	20	1.2	30	95	10	Yes	15	PT
16	(2) OR-HCM	20	1.2	30	98	10	Yes	10	PT
17	(1) OR-LCM	0.5	0.33	2.5	95–98	26.5	Yes	2.0	SP
18	(5) CR-KSM								SP
19	(2) OR-HCM	20	1.2	30	98	10	Yes	10	TT
20	(1) OR-LCM	20	0.5	30	98	10	Yes	10	PT

[1]Type of sample: Solid Samples [a](SS) Liquid Samples with high suspended solid concentrations [b] (LS-HSSC).
[2]Analytical Method:
- Open Reflux (OR): (1) OR-LCM. Low concentration of $K_2Cr_2O_7$ ($M<0.166$) (2) OR-HCM. High Concentration of $K_2Cr_2O_7$ ($M \geqslant 0.166$)
- Closed Reflux (CR): (3) CR-TM. End-point by titration (4) CR-SM. End-point spectrophotometrically (5) CR-KSM. Kits. End-point spectrophotometrically

[3]Acid-Catalyst reagent: Concentration of H_2SO_4[c]; Concentration of $AgSO_4$[d].
[4]Visualization of end-point: spectrophotometrically (SP[e]).titration: partial and total titration (PT[f]/TT[g]).

(i) Cochran's test procedure for duplicate results or the detection of outliers by differences between pairs;
(ii) precision of the analytical method used; and,
(iii) homogeneity test or test for acceptable between-sample variance.

For this purpose, 10 randomly selected distribution units of solid substrates were analyzed in duplicate and COD values were statistically evaluated.

3.1.5. Stability of samples. Materials distributed in PT schemes must be sufficiently stable over the period in which the assigned value needs to be valid. Normally, the period in question is the interval between the preparation of the material and the deadline for the return of results (one month). The material under test should be in the packaging in which it is distributed.

To ensure that the samples used in the 2nd COD-PT^{ADG} were stable, a stability study was carried out to identify if there was reproducibility of the results with time. The stability study was carried out by applying the values of *F*, which were calculated applying the one-way analysis of variance (ANOVA) of three randomly selected distribution units from the homogenization study, and it was suggested they be kept at room temperature.

3.2. Methods

3.2.1. Analytical methods

3.2.1.1. Chemical oxygen demand. The participating laboratories were free to choose the analytical method that they considered suitable for performing the COD analysis, but were advised to analyze samples using their usual techniques. Each participating laboratory was requested to make three replicate determinations, and to report the results together with a short description of the method used. Table 2 summarizes all the experimental conditions of the analytical methods used by the participants' laboratories. The studies of homogeneity and stability were carried out by the method proposed by Raposo et al. [1].

The analytical determination of COD can be classified first into two main groups [i.e. open reflux (OR) and closed reflux (CR)], and second into five methods, with percentages of each method used by the different participants in brackets:

(1) OR, low concentration of oxidant (17.5%);
(2) OR, high concentration of oxidant (47.5%);
(3) CR, end-point by titration (2.5%);
(4) CR, end-point by spectrophotometrically determination (15%); and,
(5) CR, using kits (17.5%).

The percentages of analytical methods used for OR and CR were therefore 65% and 35%, respectively.

3.2.1.2. Other parameters. Moisture, TS-dry matter and VS-organic matter were determined according to the standard methods 2540B and 2540E-APHA, respectively [7]. Fat content was determined by extraction with hexane using a Soxhlet system [8]. Protein and elemental composition were performed in a LECO CHNS-932 combustion analyzer at 1050°C, using sulfametazine as standard substrate. Theoretical oxygen demand was calculated from the elemental composition according to ISO 10707 [9]. Fiber (neutral detergent fiber, NDF) content was obtained using the method reported by Van Soest [10]. Carbohydrate content was reported by subtraction of fat, protein and lignin contents.

3.2.2. Data treatment

The internationally recommended z-score was used as the performance criteria for participating laboratories whose results were converted into z-scores according to the following equation:

$$z\text{-}score = (X_{EV} - X_{AV})/\sigma_{PT}$$

where X_{EV} is the laboratory's experimental value, X_{AV} is the assigned value (estimation of the true value of the measurand that is used for the purpose of calculating scores), and σ_{PT} is the fitness-for-purpose-based "standard deviation for proficiency assessment", defined as a target value for the acceptable deviation from the assigned value.

This means that the z-score method compares the participant's deviation from the reference value with σ_{PT},

Table 3. Homogeneity of the solid substrates. Summary of the statistical parameters obtained

Sample	Test	Experimental Value	Critical Value	Result
Gel	Cochran	0.3050	0.6020	Pass
	Precision of Method	0.39	0.5	Pass
	Homogeneity	0.00011	0.00031	Pass
SewS	Cochran	0.2603	0.6020	Pass
	Precision of Method	0.44	0.5	Pass
	Homogeneity	0.00002	0.00021	Pass
SuOC	Cochran	0.2647	0.6020	Pass
	Precision of Method	0.41	0.5	Pass
	Homogeneity	0.00001	0.00034	Pass
MB	Cochran	0.2809	0.6020	Pass
	Precision of Method	0.20	0.5	Pass
	Homogeneity	0.00004	0.00020	Pass

so the assigned value and the target standard deviation have a critical influence on the calculation of z-scores and must be selected with care if they are to provide a realistic assessment of laboratory performance.

3.2.2.1. Assigned values. In the 1st COD-PTADG, the results were too widespread to be used as a reference value based on the generally used consensus approach. In this case, the assigned values were determined on the basis of *ThOD* measurements performed at the PT coordinator's working laboratory. The same criterion was used for the 2nd COD-PT^{ADG}, but, in addition, two consensus values (mean and median) based on the results from all participants were also calculated only to estimate the degree of dispersion from the assigned value. The *ThOD*-based assigned values, mean and median consensus values for Gel and SewS solid samples were: 1236, 1201 and 1224 mg O_2 g^{-1} TS and 956, 950 and 954 mg O_2 g^{-1} TS, respectively. Similarly, the values for SuOC and MB liquid samples were: 28.164, 28.828 and 29.327 g O_2 L^{-1} and 27.793, 27.791 and 28.261 g O_2 L^{-1}, respectively. Considering the data of all the

Table 4. Summary of the data reported by participating laboratories in 2nd COD-PT^{ADG}

Lab	Sample 1				Sample 2			
	EV_{Mean} (mg O_2 g^{-1} TS)	EV_{RSD} (%)	R_{Mean} (%)	R_{RSD} (%)	EV_{Mean} (mg O_2 g^{-1} TS)	EV_{RSD} (%)	R_{Mean} (%)	R_{RSD} (%)
1	1277	3	103	3	966	1	101	1
2	1190	3	96	3	970	2	101	2
3	1142	5	92	4	815	5	85	4
4	1249	6	101	6	869	5	91	4
5	1205	2	97	2	949	1	99	1
6	1035	6	84	5	792	5	83	4
7	1219	5	99	5	953	4	100	4
8	1244	3	101	3	954	2	100	2
9	889	2	72	2	638	2	67	1
10	1235	3	100	3	954	3	100	3
11	1145	1	93	1	893	1	93	1
12	1144	6	93	6	1278	2	134	3
13	1210	3	98	3	974	1	102	1
14	1255	1	102	2	950	1	99	1
15	1245	4	101	4	871	7	91	6
16	1318	2	107	2	1004	3	105	3
17	1286	6	104	6	1057	1	111	1
18	1329	6	108	6	1093	8	114	9
19	1228	1	99	1	950	1	99	1
20	1185	6	96	6	1095	7	115	7
Lab	**Sample 3**				**Sample 4**			
	EV_{Mean} (mg O_2 L^{-1})	EV_{RSD} (%)	R_{Mean} (%)	R_{RSD} (%)	EV_{Mean} (mg O_2 L^{-1})	EV_{RSD} (%)	R_{Mean} (%)	R_{RSD} (%)
1	27567	3	98	3	33570	13	121	15
2	26853	6	95	6	26042	6	94	5
3	31527	15	112	16	27323	22	98	21
4	31433	2	112	2	31767	3	114	3
5	30512	11	108	12	28543	9	103	9
6	21665	6	77	5	22470	6	81	5
7	26476	5	94	5	26194	2	94	2
8	30233	1	107	1	27647	2	99	2
9	33519	9	119	11	28948	1	104	1
10	29451	1	105	1	28207	1	101	1
11	22647	1	80	1	22350	2	80	1
12	28700	6	102	7	30433	1	109	1
13	25467	7	90	6	25900	2	93	2
14	29963	1	106	1	29838	1	107	2
15	27933	13	99	13	23767	7	86	6
16	29255	2	104	2	28314	2	102	2
17	30553	3	108	3	29749	4	107	4
18	29399	4	104	4	27603	0	99	0
19	28566	0	101	0	28418	1	102	1
20	34850	12	124	12	32730	12	118	12

	Laboratory																			
Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	1.3	-1.5	-3.0	0.4	-1.0	-6.5	-0.6	0.3	-11.2	0.0	-2.9	-3.0	-0.9	0.6	0.3	2.7	1.6	3.0	-0.2	-1.7
2	0.4	0.6	-5.9	-3.7	-0.3	-6.9	-0.1	-0.1	-13.3	-0.1	-2.6	13.5	0.7	-0.3	-3.6	2.0	4.2	5.7	-0.3	5.8
3	-0.4	-0.9	2.4	2.3	1.7	-4.6	-1.2	1.5	3.8	0.9	-3.9	0.4	-1.9	1.3	-0.2	0.8	1.7	0.9	0.3	4.7
4	4.2	-1.3	-0.3	2.9	0.5	-3.8	-1.2	-0.1	0.8	0.3	-3.9	1.9	-1.4	1.5	-2.9	0.4	1.4	-0.1	0.4	3.6

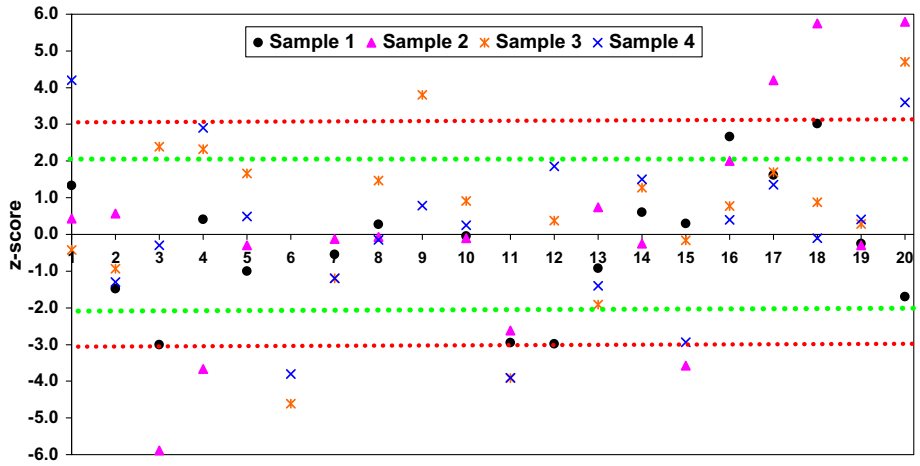

Figure 1. Overview of the z-score values obtained by laboratories participating in the 2nd COD-PT^{ADG}.

samples, it can be seen that there was a good agreement between the experimental consensus values and the theoretical assigned values.

3.2.2.2. Standard deviations for proficiency assessment. The value of σ_{PT} determines the limits of satisfactory performance in a PT scheme. It is important to note that σ_{PT} values were predefined by the PT coordinator and the criteria were communicated in advance to participating laboratories. The σ_{PT} values were determined as a percentage of the assigned value according to the appropriate form of the Horwitz equation [11], which considers the concentration level of analyte. The theoretical percentage values for GEL, SewS, SuOC and MB were 0.9%, 1.0%, 3.4% and 3.4%, respectively. However, these values were slightly modified to reflect the level of COD uncertainty in real routine work samples, so, for solid samples, the percentage was 2.5%, and for liquid samples 5.0%. These σ_{PT} values were identical to those used in the 1st COD-PTADG to prevent the different values from transferring into z-scores that could give data from different PT schemes that could not be compared.

3.2.2.3. Laboratory performance. The conventional way to evaluate the performance of each laboratory participating in a PT scheme based on z-score values was used. In the interpretation of z-scores, the following agreements were internationally made:

z-score $\leqslant \pm 2$ – satisfactory result;

z-score $> \pm 3$ – unsatisfactory result; and,

$\pm 2 >$ z-score $\leqslant \pm 3$ – doubtful result.

4. Results and discussion

4.1. Evaluation of sample-homogeneity study

Table 3 summarizes the results obtained in the statistical analysis of homogeneity data, which show that substrates selected as samples passed the statistical homogeneity tests, so they were considered homogeneous enough and suitable to be used in the PT scheme.

4.2. Evaluation of sample-stability study

The calculated F values for samples 1–4 were 0.78, 0.47, 1.72 and 2.30, respectively. All the results obtained were less than 4.96, which represents the critical F value for a confidence level of 95%. Considering that there was no significant difference between the mean values of COD determinations during the period of time established, the samples were considered stable for the study conditions.

4.3. Evaluation of laboratory performance

Table 4 summarizes the means and relative standard deviations of experimental values (EV) and recoveries (R) reported by the 20 participating laboratories. The general trend of the data reported showed that all the

Table 5. Summary of participants' results obtained for different analytical methods

Sample	Analytical Method			Average Values				Z-scores					
	Name	N^{er}	%	Mean	SD_R	RSD_R	Recovery	Z-score ⩽ ±2		±2 < Z-score ⩽ ±3		Z-score > ±3	
				(mg O_2 g^{-1} TS)	(mg O_2 g^{-1} TS)	(%)	(%)	N^{er}	%	N^{er}	%	N^{er}	%
SS-1	(1) OR-LCM	3	15	1195	56	5	97	2	67	1	33	0	0
(*Gel*)	(2) OR-HCM	11	55	1182	122	10	96	6	55	3	27	2	18
	OR-M	14	70	1185	109	9	96	8	57	4	29	2	14
	(3) CR-TM	0	0					0	0	0	0	0	0
	(4) CR-SM	3	15	1240	48	4	100	3	100	0	0	0	0
	(5) CR-KM	3	15	1241	93	7	100	2	67	1	33	0	0
	CR-M	6	30	1240	109	9	100	5	83	1	17	0	0
	Total	20	100	1201	100	8	97	13	65	5	25	2	10
SS-2	(1) OR-LCM	3	15	979	104	11	102	1	33	1	33	1	33
(*SewS*)	(2) OR-HCM	11	55	897	109	12	94	7	64	0		4	36
	OR-M	14	70	915	110	12	96	8	57	1	7	5	36
	(3) CR-TM	0	0					0	0	0	0	0	0
	(4) CR-SM	3	15	987	45	5	103	2	67	0	0	1	33
	(5) CR-KM	3	15	1080	205	19	113	0	0	0	0	3	100
	CR-M	6	30	1034	110	11	108	2	33	0	0	4	67
	Total	20	100	950	129	14	99	10	50	1	5	9	45
				(mg O_2 L^{-1})	(mg O_2 L^{-1})	(%)	(%)	N^{er}	(%)	N^{er}	(%)	N^{er}	(%)
LS-HSSC 1	(1) OR-LCM	4	20	28757	5077	18	102	2	50	0	0	2	50
(*SuOC*)	(2) OR-HCM	8	40	29347	2603	9	104	6	75	1	0	1	25
	OR-M	12	60	29150	3380	12	104	8	67	1	8	3	25
	(3) CR-TM	1	5	21665	0	0	77	0	0	0	0	1	100
	(4) CR-SM	3	15	29213	2050	7	104	3	100	0	0	0	0
	(5) CR-KM	4	20	29366	1502	5	104	3	75	1	25	0	0
	CR-M	8	40	28346	3204	11	101	6	75	1	12.5	1	12.5
	Total	20	100	28828	3204	11	102	14	70	2	10	4	20
LS-HSSC 2	(1) OR-LCM	4	20	29622	5105	17	105	1	25	0	0	3	75
(*MB*)	(2) OR-HCM	8	40	27731	1138	4	98	8	100	0	0	0	0
	OR-M	12	60	28361	2966	10	101	9	75	0	0	3	25
	(3) CR-TM	1	5	22470		0	80	0	0	0	0	1	100
	(4) CR-SM	3	15	27813	1859	7	99	3	100	0	0	0	0
	(5) CR-KM	4	20	28393	3539	12	101	2	50	2	50	0	0
	CR-M	8	40	27435	3234	12	97	5	62.5	2	25	1	12.5
	Total	20	100	27991	3027	11	99	14	70	2	10	4	20

samples were normally distributed, with a predominance of results centered on a mean value and few results in the extremes of distribution.

Fig. 1 shows an overview of all the z-scores calculated from the data reported by the participant laboratories for the four samples selected. The general impression was that the majority of reported values were satisfactory.

In addition, Table 5 summarizes participants' results obtained for the different analytical methods used. Taking into consideration the great difference in the percentages of the analytical methods used, only a relative statement could be made. However, as in the 1st COD-PT^{ADG}, no major differences in the results reported were due to the analytical method used.

It is interesting that 8 participating laboratories (40% of total) reported the four samples satisfactorily, with 62.5%, 25.0% and 12.5% of the data coming from OR-HCM, CR-LCM and OR-LCM, respectively.

The z-score performance of each sample was evaluated as follows:

- Sample 1 (Gel): 13 laboratories (65%) reported satisfactory results, 5 laboratories (25%) reported questionable results, and only 2 laboratories provided unsatisfactory results (10%).
- Sample 2 (SewS): Upon analysis, this sample showed poorer results than the solid sample (Sample 1). 10 laboratories (50%) reported satisfactory results, 9 laboratories (45%) reported unsatisfactory results, and 1 laboratory (5%) gave doubtful results.
- Sample 3 (SuOC): 14 laboratories (70%) reported satisfactory results, 2 laboratories (10%) reported questionable results, and 4 laboratories (20%) provided unsatisfactory results.
- Sample 4 (MB): The z-score values were identical to those reported for Sample 3 [i.e. 14 laboratories (70%) reported satisfactory results, 2 laboratories

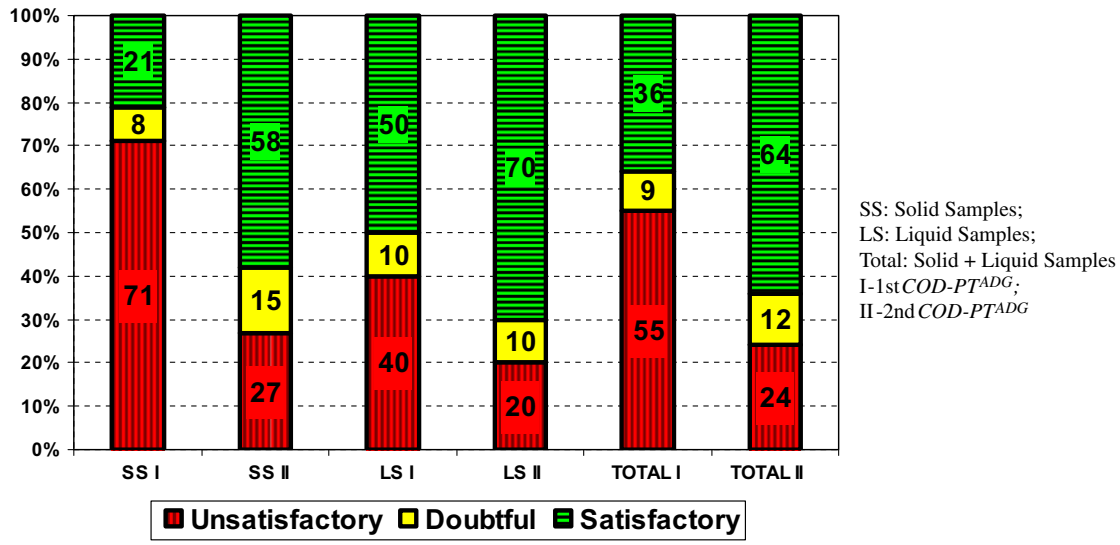

Figure 2. Comparison of z-score performance in 1st and 2nd COD-PT.

(10%) reported questionable results, and 4 laboratories (20%) provided unsatisfactory results].

The results can be outlined by the nature or characteristics of the substrate and finally grouped as total samples:

- Solid Samples: 23 z-scores (58%) were satisfactory, 11 z-scores (27%) were unsatisfactory, and 6 z-scores were doubtful (15%).
- Liquid samples with high concentrations of suspended solids: 28 z-scores (70%) were satisfactory, 8 z-scores (20%) were unsatisfactory, and 4 z-scores (10%) were doubtful.
- Total samples: 51 z-scores (64%) were satisfactory, 15 z-scores (24%) were unsatisfactory, and 14 z-scores (12%) were doubtful.

Although it is generally recognized that the analytical determination of COD samples may be ''relatively easy'' or ''relatively difficult'', it is very tempting to deduce a correlation between the type of sample analyzed and the analytical performance. For normal liquid samples (without suspended solids), the analysis of COD is considered an ''easy'' analytical determination. The results from the Aquacheck PT scheme, which ran for over 20 years, reported a percentage of acceptable results and a relative standard deviation of 91.4% and 5.8%, respectively [12]. The decrease in the overall performance of this PT scheme can be explained by considering the characteristics of the samples selected, which are potentially more difficult to analyze. However, we have no doubt that regular involvement in PT can improve the analytical performance of those laboratories taking part.

4.4. Comparisons with data from the 1st COD-PTADG

Generally, PT data are evaluated in the medium-to-long term. Although for the determination of COD in samples difficult to analyze, there have been only two PT schemes, the clear improvement in results reported could be used as ''short-term conclusions'', helping to do away with the generalized notion that solid samples and liquid samples with high concentration of suspended solids cannot be analyzed accurately, as was previously reported [13,14].

The data reported in both COD-PT schemes were summarized in terms of z-score values, and are presented in bar-chart form in Fig. 2 for graphical comparison. On the basis of the results obtained in the 2nd COD-PT^{ADG} and comparing them with the values reported in the 1st COD-PT^{ADG}, we can note that the overall performance of all participants can be considered quite satisfactory.

For solid samples, the z-scores considered unsatisfactory dropped dramatically from 71% to 27%, whilst the z-scores considered satisfactory increased from 21% to 58%. This means an improvement in the result of around 40%.

For liquid samples, the trend was also positive, with an increase in satisfactory results of around 20%.

The overall evaluation of results obtained showed that the participation in COD-PT schemes using solid samples and liquid samples with high concentrations of suspended solids improved the performance of participating laboratories by approximately 30%. This fact can be interpreted as a sign of general improvement, reinforcing the statement that the ability to produce results of acceptable quality for COD determination in ''relatively difficult'' samples seems possible.

Another indicator of the improvement in COD determination was the number of laboratories that reported the four samples satisfactorily. That 8 laboratories (40% of total) reported adequately in the 2nd PT-COD^{ADG}, compared to 2 laboratories (8% of total) in the 1st PT-COD^{ADG}, shows evident improvement.

Similar trends of overall performance improvements with participation in PT schemes were described by:

i) Whetton and Finch for some analytes of the Aquacheck PT, including COD [12];
ii) Gaunt and Whetton for analytes from alcoholic and non-alcoholic beverage industries [15];
iii) Key et al. for foods and feeds [16]; and,
iv) Earnshaw et al. for riboflavin (vitamin B_2 analysis) [5].

Nobody questions the value of PT schemes, and it is universally agreed that a well-founded laboratory must participate regularly in relevant PT. Although further research will be necessary before coming to any firm conclusion, it is foreseeable that future COD-PTs will see further potential increases in COD analytical performance, achieving satisfactory z-score values of around 90% for all the new samples distributed.

5. Conclusions

The 2nd COD-PT^{ADG} provided a valuable opportunity for evaluating the general performance of COD determination using samples considered ''difficult'' to analyze. The general performance of participating laboratories was acceptable, with 64% of the z-score values reported considered satisfactory. More significant was the improvement in results compared with the 1st COD-PT^{ADG}. Specifically, the improvement in the z-score values reported for solid samples and liquid samples with high concentrations of suspended solids was 40% and 20%, respectively. The results obtained demonstrated once more how participation in PT is successful as a way to achieve a good QC within laboratories involved in this type of chemical determinations.

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