

## **Monitoring polythionate bio-oxidation by conductivity measurement.**

### **Abstract**

Polythionates are formed by the incomplete oxidation of sulphide minerals in the grinding and flotation circuits, and particularly of those containing pyrite. Polythionate levels in the recycling water must be adjusted; otherwise, it can affect metal recoveries. The presence of different species, such as thiosulphate, trithionate, and tetrathionate in certain effluents can affect the environment. In this paper, an indirect method based on conductivity measurement for monitoring polythionate bio-oxidation is proposed. Firstly, the conductivity of acidic solutions containing polythionates is verified as the sum of the conductivities corresponding to acid and tetrathionate and, therefore, in synthetic solutions, polythionate concentrations can be estimated by simply measuring conductivity. In process water and cultures with background conductivity, polythionate concentrations are predicted from the linear relationship between polythionate concentration and the total conductivity of the system. Once the slope of the linear relationship is known, polythionate concentrations can be estimated, and therefore it is necessary to perform only chemical analysis at the beginning and at an intermediate point. The degradation of polythionates can be continuously recorded, and hence the kinetic study of a culture can be more easily and accurately performed.

### **Key words**

Polythionates, bio-oxidation, analysis, monitoring cultures, bioreactor control.

### **Highlights**

Conductivity measurement: an indirect method to determine polythionate concentration.

Conductivity measurement reveals the evolution of polythionate concentration in real time.

Conductivity helps to monitor polythionate bio-oxidation.

Conductivity measurement is useful in the control of bioreactors.

## 1. Introduction

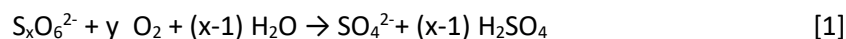
Polythionates are incompletely oxidized compounds, with general formula  $S_nO_6^{2-}$ . Polythionates appear in strongly acidic lakes near to volcanoes and in the runoff from excavated sites containing sulphur and sulphur-metabolizing bacteria (Takano et al., 1994). They are formed by the incomplete oxidation of sulphide minerals in the grinding and flotation circuits, and particularly of those containing pyrite, the most common sulphide mineral on earth. Polythionate levels in the recycling water must be adjusted; otherwise, it can affect metal recoveries (Silver, 1985).

These compounds are not considered toxic and their release into receiving waters is not regulated. However, the presence of different species, such as thiosulphate, trithionate, and tetrathionate in certain effluents can affect the environment. The polythionates are discharged into rivers and lakes where they are oxidized into sulphuric acid by the presence of bacteria. This acid causes a decrease of pH in water, thereby affecting fish and other living organisms (Miranda-Trevino et al., 2013). The severity of this effect depends on the concentration and flow rate.

In addition to the pH, other effects of polythionates on the receiving water include the reduction of dissolved oxygen concentration, the decrease in the buffering capacity, and the dissolution of metals from soil.

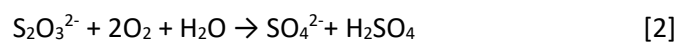
There are many routes of decomposition, depending on the pH, temperature and presence of oxidizing agents. The pH and temperature conditions in which polythionates are unstable are summarized in Vongporm, (2008).

In the literature, several possible mechanisms are laid out for the oxidation of polythionates and thiosulphate (Suzuki, 1999). All polythionates are metastable species that finally oxidize to sulphuric acid by complex reactions in series. The overall reaction is the same for all mechanisms:



$$y = 5 \text{ if } x = 5 \qquad y = (x-1) \text{ if } x = 3$$

$$y = (x-1/2) \text{ if } x = 4 \text{ or } 6 \qquad y = (x-3/2) \text{ if } x = 2$$



Extremophile microorganisms often catalyse these reactions. The presence of microorganisms improves tetrathionate oxidation, the most stable polythionate in acidic solutions (Druschel, 2003). The oxidation depends on the type of bacteria present in the medium, temperature and

dissolved oxygen conditions. *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* oxidized various sulphur compounds over a broad pH range.

Currently, the best option for the treatment of effluents containing polythionates is to place them in ponds where natural oxidation takes place. This inexpensive, simple method introduces no chemicals. Finally, generated acidity is neutralized by adding alkali in the effluent discharge. However, this passive treatment is a very slow method, which leads to long residence times (months) to achieve the desired effect. The literature contains a wide variety of proposals for oxidation based on direct and indirect photolytic methods, ultrasonic irradiation, adsorption on iron hydroxides, and use of oxidizing agents such as ozone, oxygen, hydrogen peroxide, mix of SO<sub>2</sub>-air, Fe(III)-O<sub>2</sub> (Kuyucak & Yaschyshyn, 2007; Lu et al., 2010). Although rapid, these methods are more expensive than passive treatment since they must bear the cost of reagents.

Bio-oxidation is postulated as an attractive option from a technical, economic and environmental point of view. According to Carranza et al., (2014), the continuous bio-oxidation of thiosalts in flooded packed-bed reactors, constitutes a major advance in the treatment of these effluents. Regarding the treatment in ponds, the operation time is reduced from weeks to hours. In addition, the reagent cost is reduced in comparison with active methods of treatment. Both in the design phase and during operation, it is necessary to ascertain the evolution of the determination of polythionates.

There are numerous methods of analysis for polythionate speciation, such as chromatography, electrophoresis, gravimetry, and thermal analysis or electro-analytical techniques (Miranda Trevino et al., 2013). Although these methods are of relevance, the complexity between polythionates renders them inconclusive. The polythionate concentration is usually determined by the mercuric chloride acidimetric titration method (Makhija & Hitchen, 1978). This method involves quantifying the total amount of acid produced by polythionates, expressed as tetrathionate. However, this method is time-consuming, tedious, and requires the preparation of a series of solutions and the consumption of reagents that make it overly expensive. In addition, it involves disturbing and manipulating the culture for a complete kinetic characterization. Furthermore, the method fails to enable monitoring of the evolution of polythionates in a culture. These drawbacks lead to the search for an alternative method for the indirect but rapid and continuous measurement of the polythionate concentration.

In this paper, the measurement of conductivity is proposed as an indirect method for the monitoring of the evolution of the polythionate concentration in a culture in order to follow

the kinetics of the degradation of polythionates. According to reactions [1 and 2], the polythionate oxidation increases the conductivity of the medium mainly due to the generation of acid, whereby a decrease in the concentration of polythionates involves an increase in the conductivity of the solution.

The conductivity of a solution,  $K$ , is defined as the reciprocal of the specific resistivity,  $\rho$ , or as the conductance of an electrolyte solution cube of 1 cm<sup>3</sup>. It is an additive magnitude, which means that in an electrolytic solution, both the solute and the solvent contribute, and it can be stated that:

$$K_{\text{solution}} = K_{\text{solute}} + K_{\text{solvent}}$$

Conductivity is a measure of the number of free-moving ions in a solution. For a given electrolyte, the conductivity of its solutions is a function of the solution concentration and the temperature. It depends on the number of ions per unit of volume (concentration) and also on the nature of ion (electrolyte). Since conductivity depends on the ion movements and these movements, in turn, depend on temperature, then the conductivity measurements must be performed at constant temperature.

In this paper, the specific bio-oxidation rate and polythionate degradation rate is determined by continuous conductivity measurement. The Paired Student t-Test validates the proposed method.

## 2. Materials and Methods

### 2.1 Strain

All the cultures performed in the present work are originally from a mixed culture obtained from the effluents from the Rio Tinto Mines (Huelva, Spain). This culture has been maintained for decades by successive inoculations in 9K medium (Silverman, 1959). Phylogenetic analysis shows three major phylotypes: *Acidithiobacillus*, *Leptospirillum*, and *Ferrimicrobium*. The dominant microbial species is *Acidithiobacillus ferrooxidans* \_ATCC23270 (Mazuelos et al., 2012).

For the development of the sulphur oxidation metabolic pathway of bacteria, a liquid portion of inoculum was adapted to polythionates as the only source of energy. The inoculum was centrifuged at 900-1,000 g in a Du Pont Instruments Sorvall SS 3 Automatic Centrifuge in order to remove solid particles. The supernatant was centrifuged again at 16,000 g for 5 minutes.

The decanted cells were washed and suspended in the tetrathionate medium whose composition is shown in Table 1.

Table 1: Chemical composition of the tetrathionate medium.

| <b>Salt</b>   | <b>(g/L)</b> |
|---|--------------|
| $(\text{NH}_4)_2\text{SO}_4$                                | 3.0          |
| $\text{K}_2\text{HPO}_4$                                    | 3.0          |
| $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$                   | 0.5          |
| $\text{CaCl}_2$   | 0.2          |
| $\text{Na}_2\text{S}_4\text{O}_6 \cdot 2\text{H}_2\text{O}$ | 4.0          |
| $\text{H}_2\text{O}$ (mL)                                   | 1000         |

## 2.2 Process water

The process water of a flotation plant located in Huelva (Spain) is studied. This plant treats cupriferous and polymetallic sulphide ores, which are characteristic of Iberian Pyritic Belt. The average composition of process water is shown in Table 2.

Table 2: Average composition of effluent. Concentrations are in ppm; \*polythionates expressed as tetrathionate.

|                             |       |                       |       |
|-----------------------------|-------|-----------------------|-------|
| <b>pH</b>                   | 4.85  | <b>Pb</b>             | 1.9   |
| <b>Conductivity (mS/cm)</b> | 6.50  | <b>Zn</b>             | 8.0   |
| <b>Na</b>                   | 615   | <b>F</b>              | 0.6   |
| <b>Ca</b>                   | 1,460 | <b>Cl</b>             | 306   |
| <b>Fe</b>                   | 16.0  | <b>Br</b>             | 3.9   |
| <b>Cd</b>                   | 0.1   | <b>Nitrites</b>       | 1.5   |
| <b>Cu</b>                   | 0.5   | <b>Nitrates</b>       | 21.0  |
| <b>K</b>                    | 105   | <b>Sulphates</b>      | 2,130 |
| <b>Mg</b>                   | 50    | <b>Thiosulphate</b>   | 370   |
| <b>Mn</b>                   | 4.3   | <b>Polythionates*</b> | 4,200 |

### 2.3 Synthetic liquors

Solutions of anhydrous sodium tetrathionate (Sigma Aldrich) in ultra-pure water acidified with sulphuric acid were prepared as synthetic liquors. The concentration of solutions ranges between 0 and 6 g/L of tetrathionate and the pH between 1.3 and 1.8. All laboratory material was carefully sterilized. In this same manner, solutions of anhydrous sodium tetrathionate without added acid were also prepared.

### 2.4 Analysis

The total concentration of polythionates was determined by the acidimetric method with mercury chloride (Makhija & Hitchen, 1978). Thiosulphate and sulphite ions were analysed through iodometry; discrimination between the two ions was achieved by masking the sulphite ion with formaldehyde. (Kurtenacker and Bittner, 1924; KoH, 1990).

Nitrite, nitrate, and sulphate ions were determined by ion chromatography. The 883 Basic IC plus with chemical suppression by Metrohm Suppressor module (MSM) was used for this propose.

Metal ions, F, Cl and Br were determined by inductively coupled plasma optical emission spectrometry (Agilent 715 ICP-OES).

The pH was measured by a WTW-3310 pH-meter equipped with software for multi-buffer calibration and a Sentix 41 combined glass electrode with automatic temperature compensation.

The conductivity meter was the MeterLab CDM210 (Radiometer Analytical), calibrated at 30°C and connected to a data-acquisition system. Labview 7.0 software was used for the design of a specific block diagram for continuous conductivity data collection.

## **2.5 Relationship between conductivity and polythionate concentration**

In order to correlate conductivity in terms of the acid and the polythionate concentrations in synthetic liquors, firstly, conductivity and pH were measured in acid solutions without polythionates; and secondly, conductivity and polythionate concentration were measured in solutions without added acid. These tests were carried out at 30°C.

## **2.6 Pre-treatment**

Before inoculation, the effluent of the flotation plant was pre-treated in order to eliminate any trace of thiosulphate. The pre-treatment is necessary since these authors have observed that the presence of thiosulphate in acidic solutions of polythionates is detrimental for bacterial growth (unpublished data). Without the pre-treatment, after inoculation (20% v/v), a pH close to 2 would result and thiosulphate would generate elemental sulphur and sulphite (Meyer & Ospina, 1982; Mizoguchi et al., 1976). The pre-treatment consisted of the chemical oxidation catalysed by Cu (20 mg/L) at pH 1.8 for 8 hours. During the pre-treatment, thiosulphate dismutates to sulphur and sulphite, and sulphite is subsequently oxidized by oxygen to sulphate.

## **2.7 Cultures**

Process water was inoculated with active biomass. Tetrathionate salt medium was added to attain the composition shown in Table 1 and to enrich the culture. The bio-oxidation tests were carried out in a 7-Litre bioreactor. A volume of 4.8 L of process water containing

polythionates was inoculated with 1.2 L of synthetic tetrathionate culture (20% v/v), under the following conditions: 30°C in a thermostated bath, 400 rpm of stirring speed, and aeration for oxygen saturation.

Conductivity was recorded throughout the test and at specific times, the pH and the polythionate concentration were measured. For the chemical analysis of polythionates, samples were periodically taken and filtrated through a 0.45-micron Millipore filter in order to retain bacteria and halt the bio-oxidation. This test was repeated 7 times in different cultures; in these seven cases, the polythionate concentration was determined only at the beginning and at the end of the bacterial growth.

As an example, another experiment with the same setup but at 35 ° C was performed. The evolution of conductivity was followed and chemical analysis by the Makhija method was performed in order to check the results.

## **2.8 Statistical treatment of data**

The paired sample t-test is performed in order to establish significant differences between the two methods (Montgomery & Runger, 2010). The test involves the comparison between the concentrations of polythionates obtained by the Makhija method and by conductivity measurement during the polythionate degradation in a culture. Setting up the null hypothesis ( $H_0$ ), which assumes that there are no significant differences in measurements by the two methods, assuming 5% of significance level ( $\alpha = 0.05$ ), a t-test is performed by comparing the concentration values obtained from conductivity measurements with concentration values obtained by the method of Makhija.

## **3. Results and discussion**

### **3.1 Relationship between conductivity and polythionate concentration in acidic solutions**

Figure 1 shows the conductivity vs. the acid concentration in synthetic solutions without polythionates in the pH range 1.3-2 (adjusted with dilute sulphuric acid).



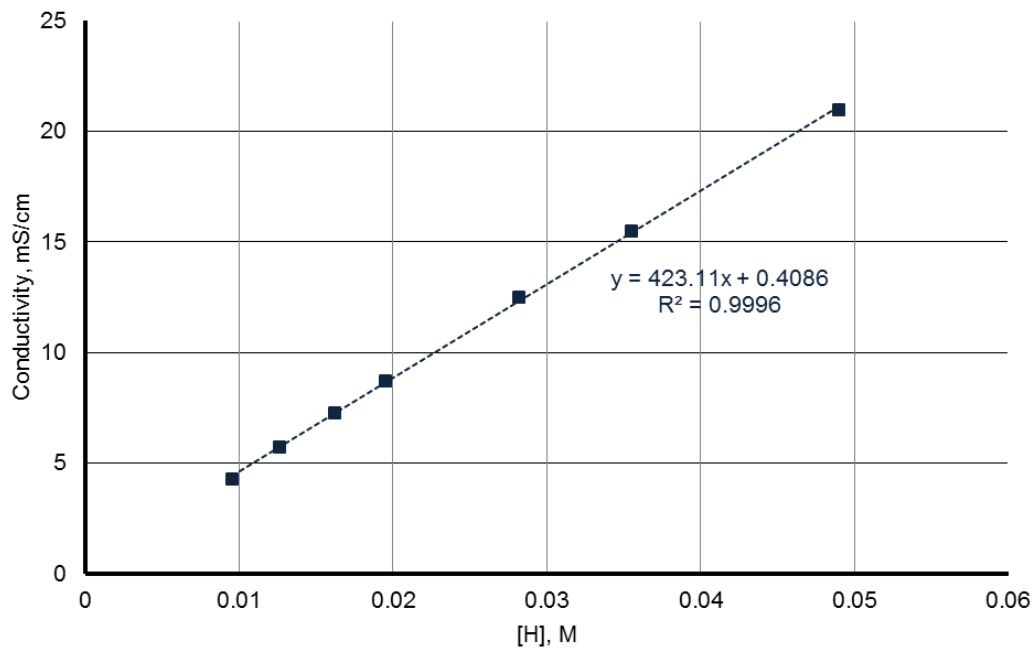


Figure 1. Relationship conductivity-[H<sup>+</sup>]. Conductivity vs. the acid concentration in synthetic solutions without polythionates, pH adjusted with dilute sulphuric acid.

Data fits a straight line with a correlation coefficient of 0.9996 and a slope of 423.11mS/(cmM).

Figure 2 shows the relationship between conductivity and polythionate concentration in tetrathionate synthetic solutions. A straight line of slope 1.0268 mS L/(cm g) is observed (R<sup>2</sup>= 0.9988).

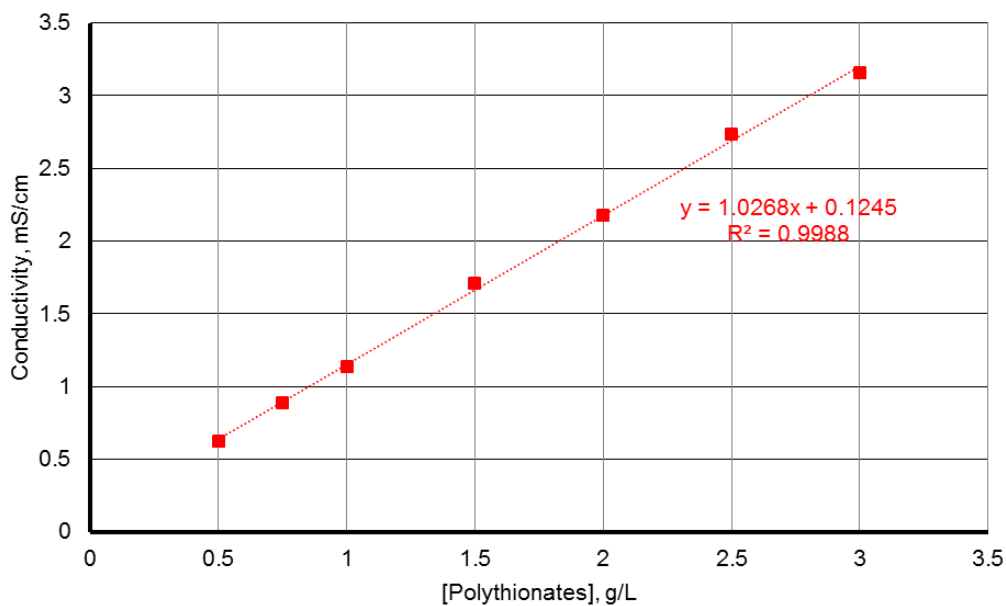


Figure 2. Relationship between conductivity and [Polythionates]

From these results, it is postulated that the conductivity of a solution that is comprised of acid and tetrathionate can be estimated from the sum of the conductivities corresponding to acid and tetrathionate, which is the sum of both straight lines (Figures 1 and 2), which results in the following equation:

$$\kappa = (10^{-\text{pH}} \cdot 423.11 + 0.4086) + (1.0268 \cdot C + 0.1245) \quad [\text{equation 1}]$$

where  $\kappa$  is the conductivity in mS/cm and  $C$  the polythionate concentration in g/L

In order to check the postulate, the conductivity of synthetic acidified solutions of tetrathionate was measured. Conductivity data, both experimental and calculated from equation 1, are shown in Table 3. In this table, both the pH and the concentration of polythionates (chemical analysis) are shown.

Table 3. Actual and estimated conductivities in synthetic solutions. \*Polythionates are expressed as tetrathionate.

| pH   | [Polythionates]<br>S <sub>4</sub> O <sub>6</sub> <sup>2-</sup> -g/L | Actual<br>Conductivity<br>(mS/cm) | Estimated<br>Conductivity<br>(mS/cm) | Relative Error<br>% |
|------|---|-----------------------------------|--------------------------------------|---------------------|
| 1.79 | 0   | 7.25                              | 7.40                                 | 1.96                |
| 1.79 | 1.54  | 8.55                              | 8.98                                 | 4.75                |
| 1.79 | 0   | 6.69                              | 6.99                                 | 4.33                |
| 1.79 | 1.54  | 8.45                              | 8.57                                 | 1.45                |
| 1.79 | 3.52  | 10.8                              | 10.61                                | -1.82               |
| 1.79 | 5.77  | 13.4                              | 12.92                                | -3.73               |
| 1.55 | 0   | 12.52                             | 12.21                                | -2.57               |
| 1.55 | 1.52  | 13.8                              | 13.77                                | -0.24               |
| 1.55 | 3.5   | 16                                | 15.80                                | -1.27               |
| 1.54 | 3.74  | 16.52                             | 16.33                                | -1.15               |
| 1.56 | 5.77  | 18.00                             | 17.85                                | -0.84               |
| 1.31 | 0   | 20.98                             | 21.26                                | 1.34                |
| 1.31 | 0.33  | 21.21                             | 21.60                                | 1.82                |
| 1.31 | 1.5   | 22                                | 22.80                                | 3.53                |
| 1.32 | 3.7   | 24.75                             | 24.58                                | -0.70               |

As expected, the conductivity of the solution is the sum of the conductivity due to polythionates plus the conductivity due to the acid. The relative error of the equation remains below 5% in all cases.

### 3.2 Relationship between conductivity and polythionate concentration in process water

In order to follow the oxidation of polythionates by conductivity measurements in inoculated process water, it is necessary to consider two factors. First, process water has a base conductivity due to the presence of other ions within the water (see Table 2); this interference can lead to systematic errors when using equation 1 to meet the polythionate values. Moreover, this basal value evolves to lower values due to gypsum precipitation. Since the amount of Ca(II) is high (about 1,500 ppm) and bacteria generates sulphate (reactions 1 and 2), the production of CaSO<sub>4</sub> is unavoidable. The second factor is the increase in total conductivity

during the polythionate degradation in a culture. This change is the balance between the increment of conductivity due to acid generation and the decrement owing to polythionate oxidation (reactions 1 and 2).

Figure 3 shows Polythionate Concentrations vs. Conductivity in a 6-litre culture of process water and 20% of inoculum at 30°C. All polythionate concentrations were determined following the Makhija method.

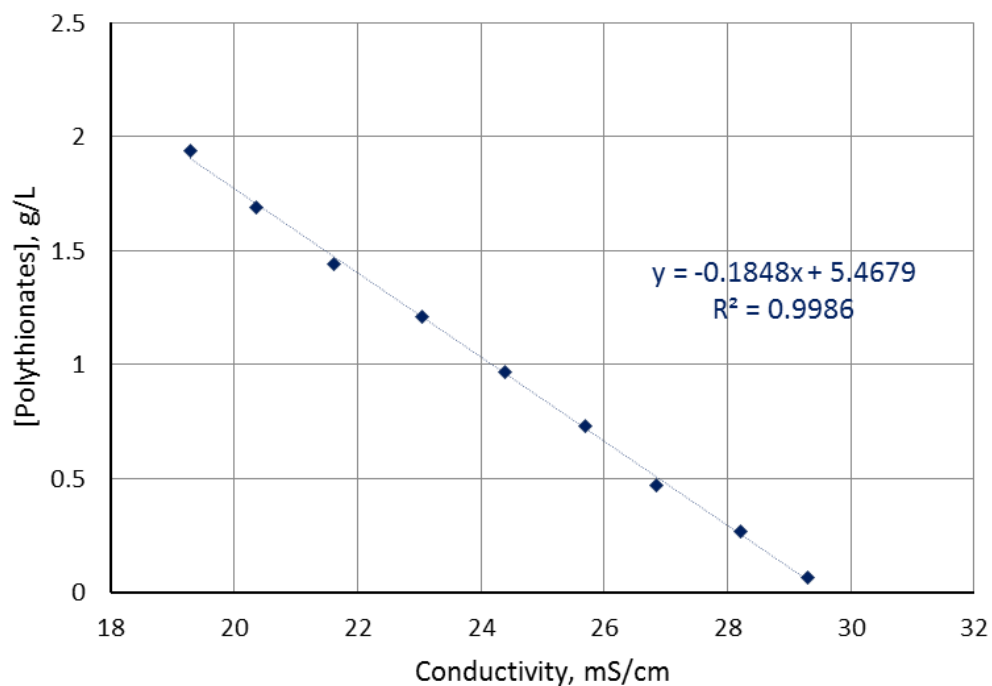


Figure 3: Polythionate concentration vs. conductivity in 6-L process water culture. Inoculum 20% (v/v); temperature 30°C. The polythionate concentration is expressed as g/L of tetrathionate.

A linear relationship between conductivity and polythionate concentration is observed ( $R^2=0.9986$ ); conductivity increases as polythionate concentration decreases. This result presents the key to determining the evolution of the concentration of polythionates over time as a function of the conductivity. The gypsum precipitation due to sulphate generation, the pH decrease due to the increase in acid concentration, and the polythionate degradation produce a net increment of conductivity. The linear relationship shown in Figure 3 was observed in all tests, which enables the polythionate concentration to be obtained as a function of conductivity by analysing only two points.

Table 4 shows the slopes for a linear relationship between polythionates and conductivity in seven tests performed under the same conditions of stirring and aeration for inoculated process water at various initial polythionate concentrations. The initial and final values of polythionate concentrations were determined by the Makhija method. It can be observed that the seven slopes are all very similar with a standard deviation of 0.0043.

Table 4: Slopes of the linear relationship between concentration and conductivity for bio-oxidation tests initiated with a variety of concentrations of polythionates.

| Culture | Conductivity (mS/cm) | [Polythionate] g/L | Slope (gcm)/mSL) |
|---------|----------------------|--------------------|------------------|
| 1       | 22.07                | 3.06               | -0.189           |
|         | 37.1                 | 0.22               |                  |
| 2       | 22                   | 2.02               | -0.1914          |
|         | 28.58                | 0.72               |                  |
| 3       | 22.07                | 3.06               | -0.1972          |
|         | 34.8                 | 0.55               |                  |
| 4       | 21                   | 2.23               | -0.1975          |
|         | 29.84                | 0.48               |                  |
| 5       | 21.2                 | 3.15               | -0.1869          |
|         | 33.24                | 0.9                |                  |
| 6       | 18.05                | 2.27               | -0.1968          |
|         | 28.21                | 0.27               |                  |
| 7       | 21.00                | 2.23               | -0.1950          |
|         | 23.00                | 1.84               |                  |

Once the linear relationship between conductivity and polythionate concentration has been demonstrated, it is only necessary to analyse the polythionate concentration at the beginning of the culture and at another point in order to obtain the slope. From the straight-line equation in the slope-point form (equation 2), the polythionate concentration can be calculated from solely the conductivity value measured. Using this equation, the continuous measurement of conductivity in culture therefore yields the concentration of polythionates remaining in the culture at any time.

$$C = m (\kappa - \kappa_0) + C_0 \quad \text{[equation 2]}$$

Where  $m$  is the slope,  $C$  is the polythionate concentration,  $C_0$  is initial polythionate concentration,  $\kappa$  is the conductivity, and  $\kappa_0$  the initial conductivity.

### 3.3 Evaluation of continuous measurement of conductivity for the monitoring of polythionate degradation

In Table 5, polythionate-conductivity value pairs, in a culture of known initial concentration (1.94 g /L) with a corresponding conductivity value of 19.30 mS /cm (starting point) are presented. From all conductivity data (continuous measurement), Table 5 shows those corresponding to discrete polythionate concentration analysis.

The slope calculated from the starting point and each one of the pairs is also shown. The analysis of variance of these two data sets is performed by application of the paired sample t-test. The paired sample t-test compares the values obtained from polythionates by conductivity method for each slope and from polythionate values measured by the Makhija method. The calculated t parameter is shown in Table 5. By comparison with the critical values of t for two tails, 2.36, the pairs marked with an asterisk (Table 5) satisfy the paired student t-test because these are lower in absolute value than the critical value. The null hypothesis can therefore be accepted and it can be said that there is no significant mean difference between the two-paired samples. This means that both methods are equally accurate.

Table 5. Slopes calculated from the starting point and each pair of values of the polythionate-conductivity and paired samples t-test.

| [Polythionate]<br>(g/L)<br>chemical analysis | Conductivity<br>(mS/cm) | Slope<br>(gcm)/mSL | Temperature<br>° C | t     |   |
|--|-------------------------|--------------------|--------------------|-------|---|
| 1.69   | 20.36                   | -0.2358            | 30.1               | -4.02 |   |
| 1.44   | 21.62                   | -0.2155            | 30.2               | -3.37 |   |
| 1.21   | 23.04                   | -0.1952            | 30.3               | -0.96 | * |
| 0.97   | 24.40                   | -0.1902            | 30.1               | 1.14  | * |
| 0.73   | 25.70                   | -0.1891            | 30.1               | 1.83  | * |
| 0.47   | 26.85                   | -0.1947            | 30.1               | -0.81 | * |
| 0.27   | 28.21                   | -0.1874            | 30.1               | 3.08  |   |
| 0.06   | 29.31                   | -0.1874            | 30.1               | 3.08  |   |

### 3.4 Conductivity method vs. Makhija method

The validation of an analytical method in reference to another can be carried out using regression techniques. In Figure 4, the values of polythionate concentration (g/L) calculated from conductivity measurements (indirect method) vs. the polythionate concentration values analysed by the Makhija method (direct method) are plotted. The four graphs have been plotted from the four slopes that meet the t-test.

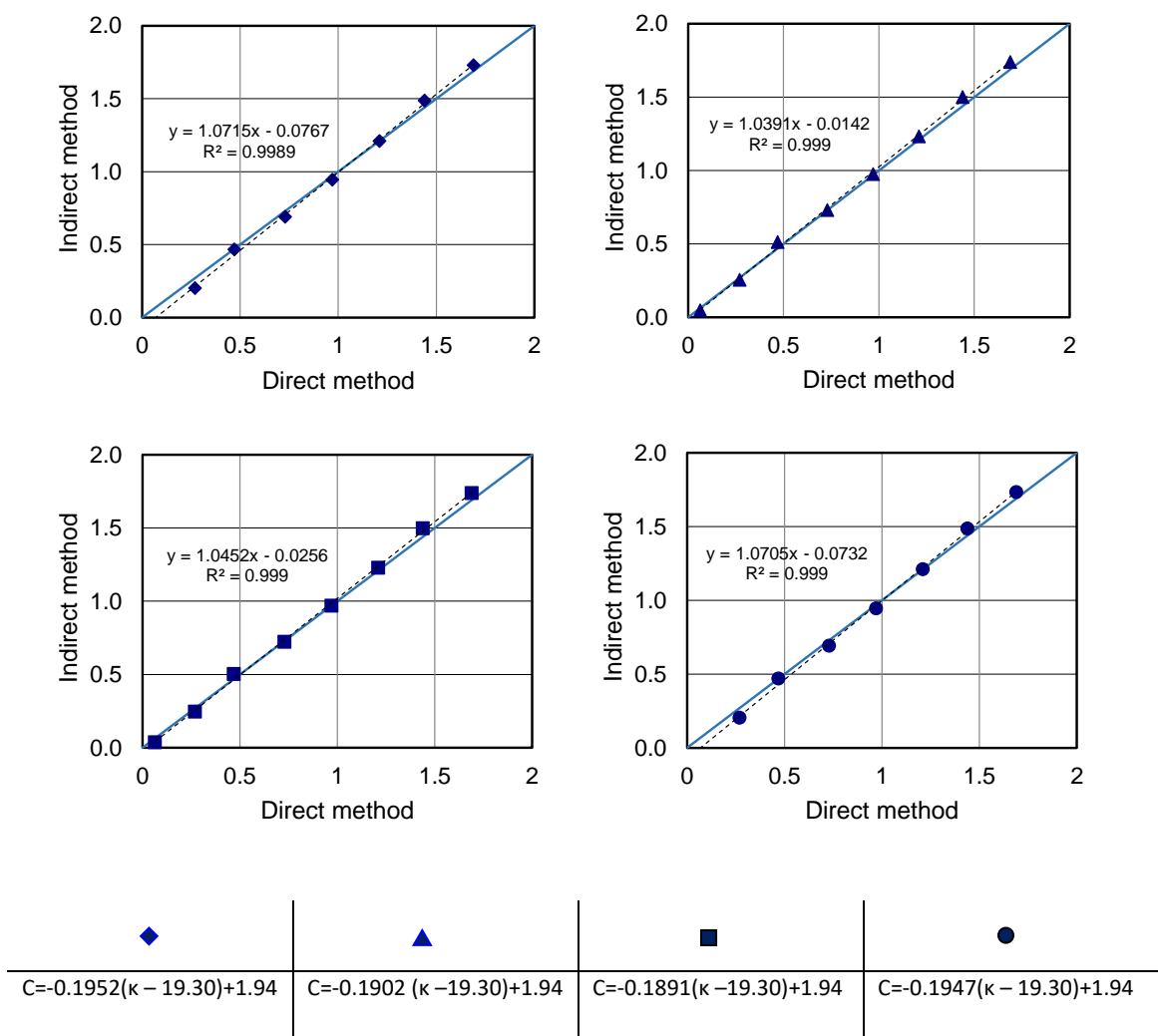


Figure 4 Comparison of direct and indirect methods for measuring the concentration of polythionates for the data that meets the t-test.

A straight-line slope of unity and a zero intercept (continuous line) express the absence of any error in the data referred to in the Makhija method. Random and systematic errors lead to a line with a slope and an intercept slightly different from the ideal (dotted lines). The observed errors may be due to the high sensitivity of conductivity with temperature.

Data whose slopes give a negative paired t-test are shown in Figure 5.

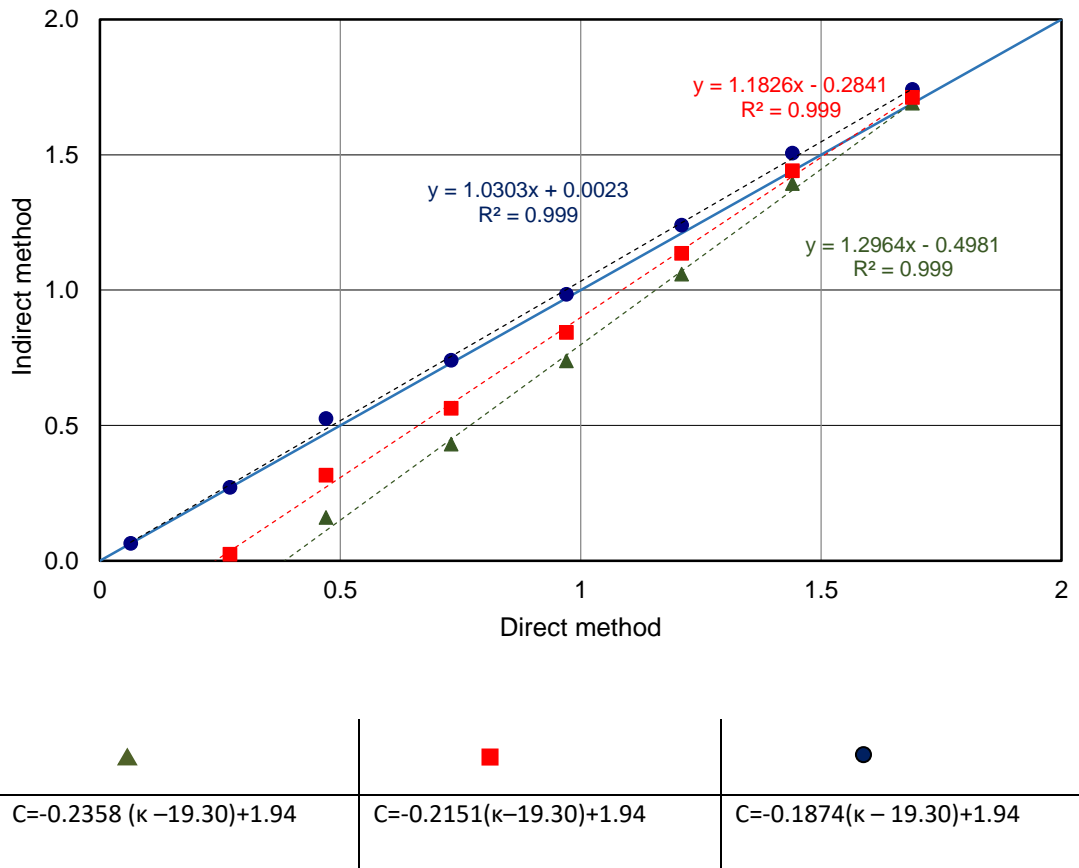


Figure 5. Comparison of direct and indirect methods for measuring the concentration of polythionates for the data that fails to meet the t-test.

Despite the similarity of the line calculated from the slope -0.1874 (circles) with the line  $y=x$  (continuous blue line), the paired sample t-test results are negative, that is, the null hypothesis for a significance level of 5% is false. Therefore, the use of concentration values that are too small to calculate the slope of the calibration line is not appropriate. This result is reasonable because, at low concentrations, the relative error of chemical analysis is very high, which leads to the calculation of an inaccurate slope.

In addition, the t-test indicates that it is not appropriate to take the second point at the beginning of the culture. In this case, the differences between the concentrations of polythionates are so small that the error of chemical analysis leads to the calculation of a slope that deviates.

In Table 6, the polythionate concentrations (g/L) obtained by each of the two methods are compared. This data corresponds to various cultures and the second point has been taken as the criterion for the calculation of the slope at a time at which the polythionate degradation reaches about 50%. The absolute error is very low in all cases, which indicates the veracity of the method.



Table 6 Comparison of the indirect and the direct method for the determination of polythionate concentrations

| [Polythionates] g/L<br>Conductivity method | [Polythionates] g/L<br>Makhija method | Absolute<br>Error g/L |
|--|---------------------------------------|-----------------------|
| 2.28                                       | 2.30                                  | 0.02                  |
| 2.23                                       | 2.17                                  | -0.06                 |
| 2.02                                       | 1.99                                  | -0.03                 |
| 1.74                                       | 1.69                                  | -0.05                 |
| 1.72                                       | 1.59                                  | -0.13                 |
| 1.50                                       | 1.44                                  | -0.06                 |
| 1.23                                       | 1.21                                  | -0.02                 |
| 1.01                                       | 0.96                                  | -0.05                 |
| 0.97                                       | 0.97                                  | 0.00                  |
| 0.72                                       | 0.76                                  | 0.04                  |
| 0.72                                       | 0.73                                  | 0.01                  |
| 0.51                                       | 0.48                                  | -0.03                 |
| 0.50                                       | 0.47                                  | -0.03                 |
| 0.25                                       | 0.27                                  | 0.02                  |
| 0.04                                       | 0.06                                  | 0.02                  |

This method provides an accuracy of 0.02 g/L (average values of the absolute errors). Therefore, a kinetic study of continuous polythionate bio-oxidation can be performed simply by following the conductivity evolution. It is only necessary to perform chemical analysis at the beginning and at an intermediate point.

### 3.5 Example of application of the analytical method.

As an example, the method is applied to a culture at 35°C in a stirred and aerated batch bioreactor. The evolution of conductivity was followed and specific chemical analysis by using the Makhija method was performed in order to check the results.

The initial concentration of polythionates was 2.30 g /L and the conductivity 20.55 mS/cm. The slope of the straight line corresponding to the intermediate point 1.50 g/L of polythionates and 24.60 mS/cm was -0.1975. Conductivity data was continuously recorded during culture and polythionate concentration values were obtained from the straight-line equation of slope -

0.1975. In this case  $t=0.34$ , which is lower than the critical  $t$  (2.77 for two tails) for a significance of 5%.

In Figure 6, the degradation of polythionates in a culture at 35°C is shown. In this figure, the polythionate concentrations, obtained by conductivity data and specific polythionate concentrations measured by the Makhija method, are both plotted vs. time. It can be observed that polythionate concentrations calculated by the Makhija method (squares) fit the curved plotted by the indirect method (blue rhombus).

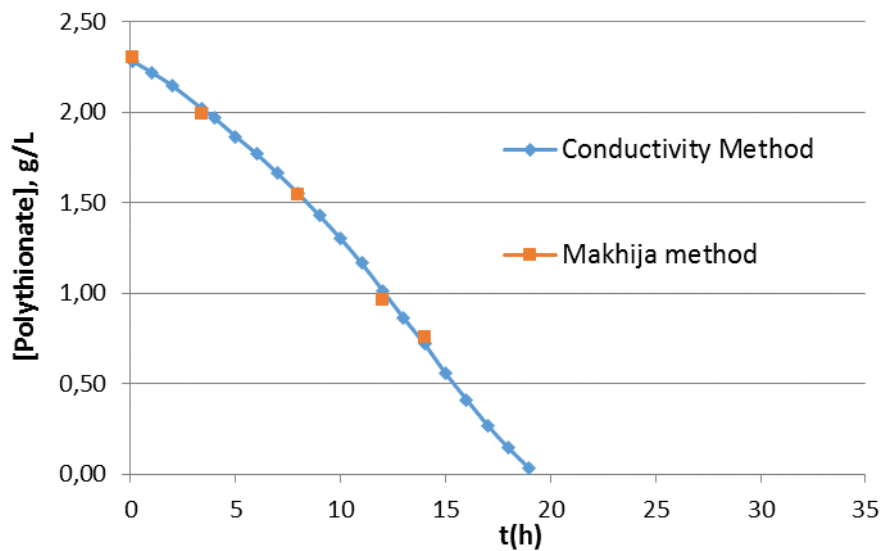


Figure 6. Polythionate degradation in a culture.  $T=35^{\circ}\text{C}$ , in a stirred and aerated bioreactor.

From the data of polythionate concentrations obtained by the indirect method proposed, the kinetics of the culture is easily studied. In this culture, the specific bio-oxidation rate calculated from the semi-logarithmic representation versus time is  $0.111\text{h}^{-1}$  and the average degradation rate of polythionates is  $0.12\text{ g/L h}$ .

The indirect method has the following advantages over the Makhija method: the degradation of polythionates can be continuously recorded, since conductivity is immediate data that is recorded at every moment; all the points of the kinetic curve are registered, which provides a more accurate characterization of the culture.

#### 4. Conclusions and considerations

In this paper, it has been demonstrated that the total polythionate concentration in cultures can be determined by conductivity measurements. This is an indirect method, which has great advantages over direct methods, such as chemical or chromatographic analysis, namely: it is simple and instantaneous; it needs neither reagents nor expensive equipment; the system remains undisturbed, thanks to the minimal sampling.

The method enables the evolution of polythionates to be ascertained in real time in cultures, in process water and in ponds.

Although this method does not discriminate between the many types of polythionates, it can be used to give information on the affinity of different types of microorganisms with effluents containing polythionates. In batch cultures performed with a single type of substrate, for example with only tetrathionate, then the kinetic information is complete.

The method is also advantageous in the control of bioreactors where continuous bio-oxidation of polythionates is performed. A change in conductivity provides warning, in real time, of an alteration in the polythionate concentrations, which enables immediate action to be taken for the proper operation of the reactor.

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