Method for Determining Organic Compound Concentration in Biological Systems by Permanganate Redox Titration

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Abstract: *An effective express method of determining organic compound concentration in biological systems (soil water extracts, culture liquid etc.) is developed. The method is based on the permanganate redox titration of carbon-containing sample in an acidic medium (pH ≈ 0) at temperature of 100°C. On the example of basic microbial metabolism of monosaccharides, alcohols, organic acids, the suitability of the method for quantitative determination of total organic carbons is shown. The method measures the rate of glucose consumption by Escherichia coli 926 (ATCC 8789) in M9 medium. We created the average calibration curve for determining carbon concentration in multi-component biological samples. The permanganate method provides high accuracy results which are comparable with the widely used phenol-sulfuric acid method. The sensitivity of the method to carbon concentration is 25 mg/l.*

Keywords*: Total carbon determination, Permanganate redox titration, Express analysis.*

Introduction

One of the most important quantitative indicators for the quality of wastewater treatment systems is the concentration of organic compounds. Taking into consideration the operating mode of treatment facilities, express method for determining this parameter is required. In analytical chemistry quantitative determination of the organic compounds concentration by dichromate oxidation is widespread [1]. The obvious disadvantages of this method are the need for a prolonged boiling of analytical samples on a sand bath, the use of a flow-through water cooler, as well as the residual dichromate titration by Mohr's salt. As an alternative to standard methods we consider permanganate redox titration for rapid and efficient determination of carbon's concentration in biological systems. The method is based on a cohesive reaction (1) of organic compounds oxidation to end products (CO_2, H_2O) and the bounded reduction of Mn^{7+} to Mn^{2+}.

$$C + MnO^-_4 + 4H^+ \rightarrow CO_2 + Mn^{2+} + 2H_2O \quad (1)$$

This method is used in analytical chemistry for determining reduced compounds concentration in non-biological systems [1]. Permanganate ion has several advantages compared to chromate ion:

1) Standard potential of MnO^-_4 400 mV higher than that of $Cr_2O_4^{2-}$ (E_0 equals +1200 and +800 mV, respectively) [5]. Due to strong oxidizing ability MnO_4^- provides high speed of oxidation and can be used for redox titration of various organic compounds within wide concentration range. This property makes MnO_4^- multifunctional towards organic sample and strongly decreases analysis time.

2) Permanganate ion is not only an oxidant, but also a redox indicator. Oxidized MnO^-_4 ion is colored purple while reduced Mn^{2+} ion is colorless. During carbon-containing sample titration by permanganate MnO^-_4 ions are reduced and decolorized. The oxidation of carbon and reduction of Mn(VII) occurs at a stoichiometric ratio. The concentration of organic compounds is determined by the amount of MnO^-_4, used for the titration.

In case of complete organic compound oxidation the analyzed solution acquired violet color. This signals the end of titration reaction. This avoids the excessive amount of titrant and subsequent determining of its residual concentration which is an additional advantage compared to the dichromate method. These two advantages of the permanganate ion reduce both the time of analysis from 3-6 hours to 10 minutes, as well as the of reagents and analytical operations.

pH value significantly affects the level of MnO^-_4 oxidation ability (measured in Eh value) and end products of Mn^{7+} reduction [8]. At alkaline pH permanganate oxidizing ability is relatively low and equals the standard potential of MnO^-_4 of +560 mV. Reduction reaction is incomplete, as end products are Mn^{6+} compounds. At neutral and slightly acidic pH E_0 = +800 mV, the reduction reaction is complete with the end product being Mn^{2+} (Eq. (2)). Yet the end product is MnO_2 – an insoluble brown suspension.

$$2\ MnO_4^- + 3Mn^{2+} + 2H_2O = 5MnO_2 \downarrow + 4H^+ \quad (2)$$

This complicates the identification of the purple color in the MnO^-_4 / Mn^{2+} system. Rapid and complete oxidation of most soluble organic compounds occurs only in a strongly acidic medium (pH ≈ 0). At low pH value standard potential of MnO^-_4 is the highest and equals +1200 mV. The end reduction product is a colorless Mn^{2+} ion which does not prevent the identification of MnO^-_4 purple color (Eq. (3)):

$$MnO_4^- + 8H^+ + 5\bar{e} = Mn^{2+} + 4H_2O \quad (3)$$

The aim of our work was adapting the permanganate redox titration method and creating an express method for determining the organic compound concentration in biological systems, as well as demonstrating its reliability. The essence of the method is modification is the titration of organic compounds at 100°C by a standard solution of potassium permanganate ($KMnO_4$) in acidic medium (H_2SO_4) [1, 5]. High temperature strongly affects the process kinetics and leads to instantaneous oxidation of organic compounds. Compared to HCl and HNO_3 we consider sulfuric acid to be preferable for sample acidification. These acids are evaporated during heating which results in their concentration being decreased in the reaction mixture. In addition Cl^- can be oxidized by MnO^-_4 which distorts the results of the sample titration.

Materials and methods

For redox titration we used aqueous solutions of the main classes of organic substances: carbohydrates (glucose), alcohols (ethanol, glycerol), organic acids (oxalic acid, citric acid) within a concentration range of 25-500 mg/l. In a chemically clean tube 1 ml of an organic compound solution and 0.1 ml of concentrated sulfuric acid were transferred. The sample was

heated on open fire or water bath to 100°C (5 minutes). Standard (0.1%) water solution of $KMnO_4$ (0.05 ml aliquots) was added to heated sample. The solution was titrated until a light-purple color typical for permanganate ion appeared. The coloring remained constant for at least 1 hour. To avoid ethanol evaporation during analysis, titration was carried out in hermetically sealed vials. Permanganate solution was added to the vial via a syringe. pH was monitored by a pH meter "pH 150 mA" and a pair of electrodes (measuring electrode EPV-1 and silver chloride electrode-comparison EVL-1M3).

For a quantitative determination of total carbon in a biological system we used culture medium of *E. coli* 926 (ATCC 8789). The strain was grown on the inclined surface of HiMedium agar for a 24-hour period and suspended in sterile 0.9% saline, transferred to M9 medium and cultivated during 24-hour period [7]. The M9 medium contains (per 1 liter of distilled water) Na_2HPO_4 – 6.0 g, K_2HPO_4 – 3.0 g, NaCl – 0.5 g, NH_4Cl – 1.0 g. Upon autoclaving 10 ml of 0.01 M $CaCl_2$, 1 ml of $MgSO_4$ solution and 5.0 ml of 40% glucose solution were added. Then the strain was grown in strictly aerobic conditions on M9 medium during 8 hours at 30°C on a shaker (360 rpm/min) in hermetically sealed vials (500 ml). In order to ensure cultivation we added 0.1 ml of inoculum to the nutritional medium to reach 0.1 opt. units. Controlled parameters were biomass gain, gas phase composition, and the concentration of total carbon and carbohydrates in the supernatant of the culture liquid. These parameters were measured every hour during the 8 hours of cultivation time. The optical density of the medium was measured colorimetrically by CPC-2PM, λ = 540 nm, the optical path length – 0.5 cm. Gas phase composition was determined according to a standard procedure on the gas chromatograph LCM 8MD [2]. This chromatograph is equipped with two steel columns – one (I) for analysis of H_2, O_2, N_2 and CH_4, the second one (II) – for CO_2.

The concentration of gases in the vial with a growing culture of *E. coli* – H_2, CO_2, N_2 and O_2 (in %) was calculated according to the standard procedure by the peak area [4]. Phenol-sulfuric method is the generally accepted one for determining carbohydrates concentration [2]. Therefore, we determined the concentration of glucose with both permanganate and phenol-sulfur methods in order to validate the method of permanganate redox titration. A culture liquid sample for redox titration was prepared as follows. An aliquot (5 ml) was centrifuged by the benchtop centrifuge OPn-8 (15 min, 5000 rpm). After cells deposition 1 ml of supernatant was collected. In order to determine the glucose concentration by the phenol-sulfuric method 0.5 ml of a 5% phenol solution and 2.5 ml of concentrated sulfuric acid were added into the vial containing 0.5 ml of a sample. The intensity of the coloring was determined photocolorimetrically by CPC-2PM at λ = 490 nm, the length of optical path – 0.5 cm [3, 9].

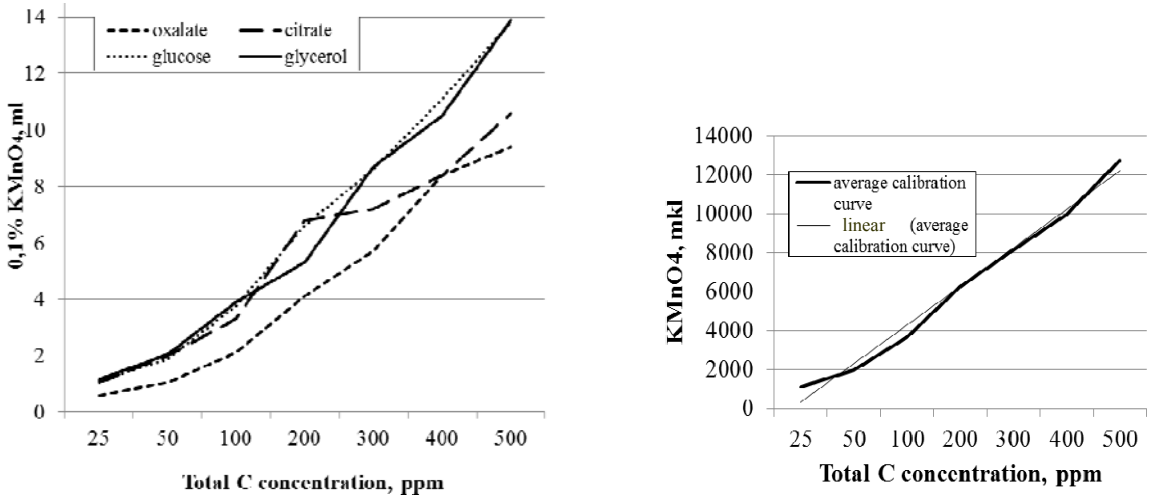
Results and discussion

We showed suitability of modified permanganate redox titration method for determining the concentration of total carbon in the most widespread classes of organic compounds used for heterotrophic microorganism cultivation (oxalate, citrate, glucose, glycerol).

The idea of such express-method belongs to Prof. Tashyrev. The primary analysis was made by Dr. Matveeva and Dr. Tashyreva. Advanced analysis and the experiment cycle were made by Ph.D. students Suslova and Govorukha, and Dr. Brovarskaya.

Fig. 1 displays graphs of organic substance titration with potassium permanganate. Fig. 1A shows that carbon concentration in a sample is directly proportional the amount of

oxidant ($KMnO_4$) necessary for a complete reaction within the concentration range of 25 to 500 mg C/l for all classes of organic compounds. We determined the sensitivity limit of permanganate redox titration method. It is 25 mg C/l. Thus, the results can be used as the calibration curves displaying the determination of total carbon in monosaccharides, organic acids and alcohols.



A) B)

Fig. 1 Graphs of organic substance titration with potassium permanganate
A) calibration curves for oxidation of carbon-containg compounds by potassium permanganate; B) average calibration curve for determining carbon concentration in organic compounds (glucose, sodium citrate, glycerol).

However, the volatility of ethanol led to distortion of the results. This is due to the fact that the organic compound remains in the gaseous phase and oxidizer – in the liquid one. Therefore, the oxidation reaction takes place only at the interface of liquid and gas states. That is why during redox titration volatile organic compounds need to be shaken in hermetically sealed vials. Based on these calibration curves we created an average calibration curve for determining carbon concentration in the multi-component biological samples (Fig. 1B).

It was necessary to compare the results obtained by redox titration with those obtained by another commonly used method in order to confirm the suitability of our modified method of determining total carbon concentration in biological systems. We used phenol-sulphuric method, as it is a commonly accepted one for determining glucose concentration [9]. The main task was to ensure such conditions of cultivation in which glucose was the only organic carbon-containing component. This ensured high measurement accuracy and consequently allowed validating the permanganate redox titration method. To that end, we have used a model biological system – the dynamics of glucose consumption during the cultivation of *E. coli* 926 (ATCC 8789) on a standard synthetic medium M9. Under strictly aerobic conditions, glucose is completely oxidized by *E. coli* to CO_2 and H_2O. This was achieved by an excess ratio of air and nutrient medium in vials. O_2 concentration was monitored by gas chromatography. As the criteria for strictly aerobic conditions and complete oxidation of glucose we used consistently high oxygen concentration and the absence of hydrogen as a gaseous product of anaerobic glucose fermentation.

Fig. 2 shows the data of the gas phase composition during the *E. coli* cultivation. O_2 concentration was maintained at the high level within the range of 19.56-21.03% and was

not decreasing during the overall cultivation cycle, H_2 synthesis was not observed. This serves as evidence of strictly aerobic conditions.

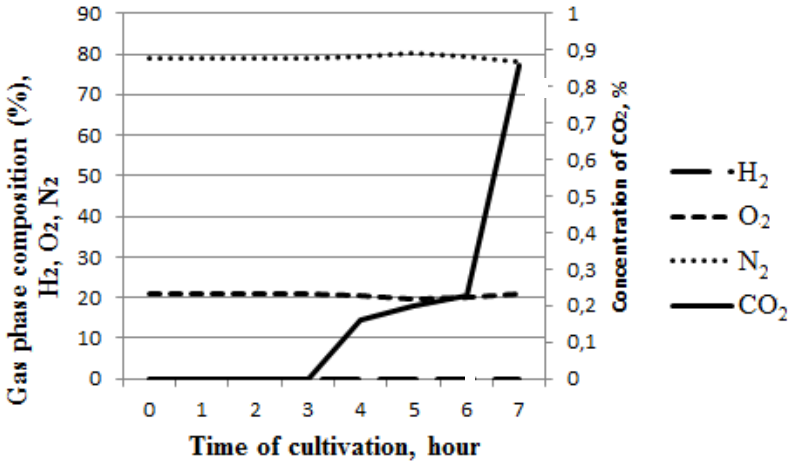


Fig. 2 The changes in gas phase composition during *E. coli* 926 cultivation

Production of CO_2 started from the third hour that testified to glucose consumption, increased up to the seventh hour of cultivation and reached 0.86%. Fig. 3 shows the growth dynamics of the *E. coli* 926 (ATCC 8789) and the data on the decrease of total carbon concentration in the culture medium. The decrease in glucose concentration was measured by both the permanganate and phenol-sulfuric methods.

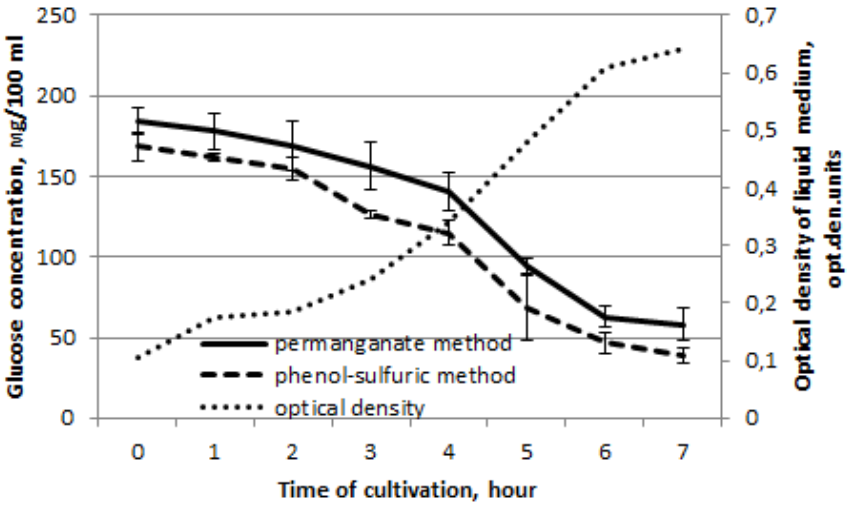


Fig. 3 Dynamics of change in total carbon concentration during the growth of *E. coli* 926 on M9 medium

Graphs of permanganate and phenol-sulfuric methods are rather similar [4]. Furthermore, both permanganate and phenol-sulphuric methods show a dramatic decrease in organic compounds concentration, between the 3rd and 7th hours of cultivation. This coincides with a significant increase in optical density (Fig. 3) and active CO_2 formation (Fig. 2). This confirms the accuracy of the results obtained in a model biological system of *E. coli* on M9 medium and the efficiency of the permanganate redox titration method for the determination of carbon concentration in biological media.

Conclusions

This method can be used not only in analytical chemistry but also for determining the total concentration of organic compounds in biological systems. The method is suitable for determining the concentration of carbon compounds that are accessible for the majority of chemoorganotrophic organisms – monosaccharides, alcohols, organic acids. It can be used for analytical determination of total carbon concentration in nutrient media and complex biological systems (soils water extracts, culture liquid of microorganisms etc.) and soluble organic compounds in wastewater as well. The advantages of this method are its high speed high rate and simplicity, availability of materials, the possibility of determining total carbon within a wide range of organic compounds. The method is furthermore suitable for a rapid determination of total carbon concentration under field conditions and in biotechnological installations – treatment plants, industrial wastewater etc.

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