Determination of organic carbon in soils and sediments in an automatic method

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Abstract: Our automatic digestion device is applied in determining the quantity of organic carbon in the soils/sediments. Its operation process is simple. The reaction conditions are optimized; the complex pretreatments are automated; and a great number of samples can be analyzed at the same time. Comparison shows that the experiment using the device is safer and easier. The correlation coefficiency is greater than 0.999, indicating a good linear relationship. The relative standard deviations of three different concentrations are less than 5%. Standard addition recoveries of high and low concentration range between 94.7% and 100% and between 91.7% and 105% respectively. Method determination limitation (MDL) of this method meets the practical requirements. The device in this paper supports a compositive SOC determination method. Its advantages include improved time and labor efficiency, and accuracy. The device is widely used in the studies of agricultural science, carbon cycle, climate change and environmental protection.

Keywords: Automatic digestion; Soils; Sediments; Organic carbon

Introduction

Organic carbon is widely distributed in soils and can be found in a variety of materials, from simple sugars and carbohydrates to complex macromolecular protein, fat and organic acids (Schnitzer M, 1999). The content of soil organic carbon (SOC) is the criterion for differentiating the input generated by biological residues from that generated by other organic materials. The loss of SOC is caused by microbial decomposition of organic materials. It is usually taken as an important index in the evaluation of soil fertility (Parton W J *et al.* 2015). The content of SOC in the soil is generally around 5%. The higher the content of SOC, the stronger the soil's ability to absorb contaminants (Rytwo G *et al.* 2010).

SOC has a crucial influence on the migration and transformation of pollutants in soils. Studies show that SOC in soils can combine with pollutants such as heavy metals and organic pollutants, changing their physical and chemical properties. Such a process eventually changes the pollutant migration, enabling the pollutants to migrate in soil from the surface layer to deeper layers, or, with air, to groundwater or surface water, causing secondary pollution. Owing to its large amount, minor changes in SOC have the potential to affect the carbon cycle, resulting in greenhouse effect, global climate change and thus greatly influence the distribution, composition, structure and functions of the terrestrial ecosystems (Sierra C A *et al.* 2015). In the study of environmental evolution, the content of organic carbon in soils is considered one of the most important indices of climate change. Therefore, the determination of SOC content is of great significance in controlling soil pollution, protecting soil quality, and maintaining ecological balance.

Methods of determining the content of SOC include spectrum calculation, titrimetry, TOC instrument, element analyzer measurement and linear regression of particulate matter (Avramidis P *et al.* 2015; Chen J *et al.* 2015; Kuang B *et al.* 2015; Rodionov A *et al.* 2015; Schrumpf M and

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Kaiser K, 2015; Stevens F *et al*. 2015). However, these methods are highly complicated, time-consuming and at the risk of serious errors. To improve the efficiency, accuracy and automation level and more importantly, simplify the experiment procedures, this study uses the automatic digestion analyzer, a method suited for determining SOC content in samples in large quantities.

Experiment

Reagents and materials. The reagents and their places of purchase are listed as follows:
-Glucose (Tianjin Chemical Reagent Factory, AR)
-Potassium dichromate (Beijing Chemicals, GR)
-Mercury sulfate (Beijing Jinxing Chemicals, AR)
-Sulfuric acid (Beijing Chemicals, GR)

Take 80 g of potassium dichromate and dissolve it in an appropriate amount of water. After dissolution, move the solution to a 1 000 mL volumetric flask and dilute to scale with ultra pure water. Potassium dichromate solution ($K_2Cr_2O_7$) with a concentration of 0.27 mol/L is acquired.

Take 10.00 g of glucose and dissolve it in an appropriate amount of water. After dissolution, move the solution to a 1 000 mL volumetric flask, and dilute to scale with ultra pure water. Standard liquid glucose ($C_6H_{12}O_6$) with a concentration of 10.00 g/L is acquired.

Take 1.00 g of mercuric sulfate and dissolve it in an appropriate amount of sulfuric acid. After dissolution, move the solution to a 1 000 mL volumetric flask and dilute to scale with sulfuric acid. Mercuric sulfate solution with a concentration of 10.00 g/L is acquired.

Equipment. The equipment and their places of purchase are listed as follows: spectrophotometer (ShimadzuUV-2600), balance (Mettler AE163), centrifuge (Beijing JingliLD4-8), soil sieve (60 mesh), and automatic digestion analyzer (Fig. 1). Existing commercial automatic chemical analyzer (AMS from Italy or France) can be used to measure chemical elements, salts, lysine and urea. Automatic digestion devices, such as Auto DigiBlock S60 (Labteach) and ST-60 (Polyteach), that measure metal elements in soils/sediments are also widely used. However, automatic devices that measure organic carbon in soils/sediments are not yet developed. Our device is thus designed to address this problem. The storage bottles and tubes for glucose and ultra pure water are kept in dark so that bacteria won't grow. The other two storage bottles and tubes are made from Teflon. Their inside is kept smooth to avoid possible crust blocking the tube.

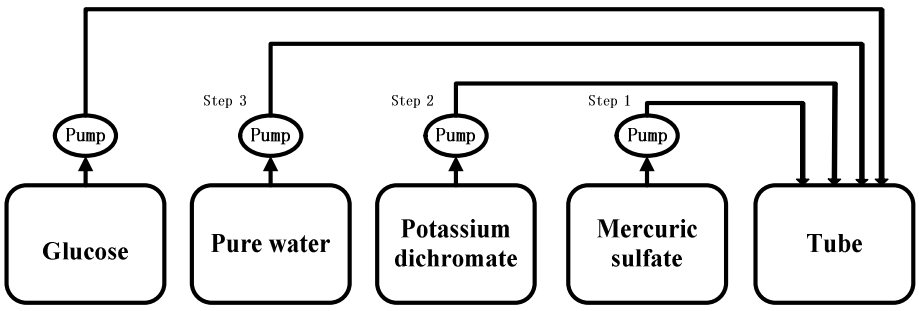

Fig. 1 Diagram of automatic digestion

Method. Sample: the soil/sediment samples are put in clean white enamel tray, split into 2 to 3 cm layers. Plants, insects, rocks and other residues are removed, and then clods are crushed with faller hammer. Samples are air-dried and stirred while being dried. According to the quartering method, 10 g to 20 g of fine ground soil are sieved through the 60-mesh soil sieve (0.25 mm). 0.5000 g soil sample is put in 100 mL of PTFE tube for testing.

Calibration curve: 0.00 mL, 0.50 mL, 1.00 mL, 2.00 mL, 4.00 mL, and 6.00 mL of glucose solutions (10 g/L) are measured and put into 100 mL PTFE digestion pipes. The corresponding amount of organic carbon are 0.00 mg, 2.00 mg, 4.00 mg, 8.00 mg, 16.0 mg, and 24.0 mg. Soil samples and the calibration curve are put in the automatic digestion analyzer. Automatic digestion analysis is set up, as is shown in Table 1.

Table 1 Automatic digestion procedure

Order		Program
1	Add reagent	Mercuric sulfate solution, 10 mL
2	Add reagent	Potassium dichromate solution, 5 mL
3	Vibrate	1 minute
4	Heat to	135 ℃
5	Heat	135 ℃, 30 minutes
6	Vibrate	1 minute
7	Cool	30 minutes
8	Constant volume	Pure water, 100 mL

The digestion tube is cooled and put in centrifuge at the speed of 2 000 r/min for 10 min. Supernatant on wavelengths of 585 nm is compared with water in 10 mm cuvette. Absorbance is measured respectively. With absorbance as the ordinate and the corresponding amount of organic carbon (mg) the abscissa, the calibration curve is drawn to calculate the amount of organic carbon in the soil samples.

Results and discussion

Operation process. Automatic digestion analyzer is highly efficient. During preparation stage, it takes less than half a day to make solutions with strong acidity and oxidization. Then, the time spent weighing the samples depends on the number of samples. After preparation, the samples in tubes are set on a shelf as soon as the device begins to run. Calibration curve is created by the pump connected with the container of glucose solution. It measures different volumes of solutions in different tubes and then forms a calibration curve. All pumps are calibrated before use so that solutions can be added accurately. Mercuric sulfate solution is added to provide Hg^{2+} as the screening agent of Cl^-. Its importance will be explained in the next section. The main concern is that it's toxic to the environment. After eliminating Cl^-, the interference, the pump of potassium dichromate solution starts running. The new chemical is mixed with the solution and soil, engaging in a vigorous reaction. Then, vibration blends the system thoroughly. As temperature and reaction time significantly affect the process, the system is heated to 135 ℃ and kept at 135 ℃ for 30 min. The tube is vibrated during the digestion when necessary. Eventually, solution cools down and its volume is diluted to 100 mL. A sensor controls the final volume. Subsequent operations are the same as described above in the manual procedures.

Confirmation of the analysis condition. The common method of potassium dichromate oxidation is based on Walkley-Black process (Gelman F *et al*. 2012) as equation (1) shows. The recovery rate of this reaction is relatively low at room temperature, due to incomplete digestion. Thus, heat treatment is adopted. The higher the temperature is, the more thoroughly they will react. In principle, the experiment temperature can be as high as possible. However, to avoid the decomposition of potassium dichromate solution, the temperature cannot exceed 150 ℃, otherwise it loses its oxidizability as Equation (2) shows. 135 ℃ is considered an appropriate temperature.

$$2Cr_2O_7^{2-} + 3C_0 + 16H^+ = 4Cr^{3+} + 3CO_2 + 8H_2O \quad (1)$$

$$2K_2Cr_2O_7 = 2K_2Cr_2O_4 + 3O_2 \quad (2)$$

There are some disturbances during the experiment. Substances, such as chloride and Fe^{2+} can cause positive errors as they consume potassium dichromate, while MnO_2 can bring about negative errors. Besides, Fe^{2+} is oxidized when air-dried and Cl^- is removed after reacting with mercuric sulfate. Also, MnO_2 is part of the reaction with potassium dichromate. However, as the amount is small, disturbances are negligible.

Method advantages. Most researchers in soil science are confronted by the same problem when abrading samples. For analytical experiments, the size of soil grains is required smaller than 100 mesh. However, in this method, 60 mesh is enough.

If the Walkley-Black process is done manually, the grinding and solution making will be time consuming. Preparing more than five standard samples, weighing samples and mercuric sulfate, adding potassium dichromate solution and sulfuric acid and setting the volume after heating are five inevitable steps. Each step takes at least 2-5 min per sample. One sample usually takes more than 1 hour. Moreover, all the chemicals used in this experiment are dangerous. A more efficient way of processing is sought after. And the automatic digestion analyzer can greatly simplify the procedures. The researchers can simply set up the device and get the results a few hours later. This method not only saves time, but also guarantees experiment safety.

For experiment methods, accuracy is crucial. Among the many methods mentioned in Introduction, chemical methods are by far the most accurate and most other methods are used to estimate, analog or forecast. Each step of chemical methods aforementioned could result in errors. For manual operation, if every step has a systematic error of approximately 1%, the total would be 10%. But using the automatic digestion analyzer, the only error that may occur is when weighing soils. The automatic digestion analyzer reduces human interference, thus improving accuracy, parallelism and repeatability. The pumps and sensors will influence accuracy, and they need to be carefully calibrated before use.

Linear, precision and accuracy. In order to reduce experimental errors, sample masses should be over 0.1 g. Considering that regular soils contain less than 5% of SOC, it is unnecessary to set the calibration curve too high. If there is a sample of high SOC content, we can either decrease its quantity or increase reagents. When increasing reagents, standard samples should be processed in the same way as the high-content sample. Fig. 2 shows the relativity between absorbance and the mass of SOC. Correlation coefficient is greater than 0.999, indicating a good linear relation. The bottom and top points of the curve is respectively 0 and 24 mg. This range covers the content of most samples.

Three standard samples of different masses are experimented on and the results are listed in Table 2. All three's RSD is lower than 5%. The tendency of RSD is consistent with that in other methods–it has a negative correlation with concentration.

In recovery test, a 0.5 g soil sample contains 4.67 mg SOC. 2 mg and 20 mg glucose is added into the sample and this is repeated 6 times. All recoveries range between 91.7% and 105%, as is shown in Table 3. The recovery of 20 mg sample is weaker than that of 2 mg sample, in that 20 mg is substantially greater than 4.67 mg. Theoretically, the mass of addition should be close to the sample's mass, but it is rare to find a soil sample with such a high SOC content.

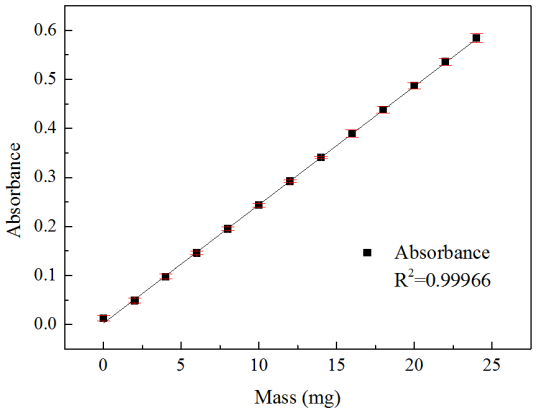

Fig. 2 Calibration curve of the method

Table 2 Precision data of three concentrations

Mass of SOC (mg)	1	2	3	4	5	6	Average (mg)	SD	RSD (%)
1	1.02	0.96	0.97	1.06	0.94	1.03	1.00	0.05	4.69
10	9.60	10.2	10.3	9.60	10.3	9.50	9.92	0.39	3.90
20	20.5	20.4	19.6	19.9	19.7	20.2	20.1	0.37	1.86

The MDL is confirmed in EPA method (US EPA, 1992). Low-concentration standard samples are tested 7 times. The concentration is generally 1 to 3 times greater than MDL. Thus MDL equals 3.143 times SD. The data are listed in Table 4. The MDL meets the analysis requirements.

Application. Currently, the device can digest 60 samples at the same time. To digest each sample, four reagents are added. Adding one reagent takes 10 seconds, and digesting one sample takes 40

seconds. 60 samples approximately take 40 minutes to process. Then, samples are heated for 30 minutes and cooled for 30 minutes before they are put into constant volumes. The next step is colorimetry, which can only be done with human eyes. In this step, one sample takes 1 minute, and 60 samples need an hour in total. The process is facilitated with plenty of cuvettes. With the new device, analyzing 60 samples takes 2.5 hours in total. However, with a TOC analyzer, using combustion method, it takes 10 to 20 minutes to analyze one sample. Taking pretreatment to eliminate inorganic carbon into consideration, 60 samples need at least 2 to 3 days to analyze.

Table 3 Recovery data of two concentrations

Mass item	2 mg		20 mg	
	Measured value (mg)	Recovery (%)	Measured value (mg)	Recovery (%)
1	6.61	97.2	24.2	97.7
2	6.67	100	24.5	99.2
3	6.72	102	23.1	92.2
4	6.78	105	24.1	97.2
5	6.61	97.2	24.7	100
6	6.50	91.7	23.6	94.7

Conclusions

With the development of mechanical technology, new advanced devices are coming into use. They can meet the requirements of analysis and greatly improve the process. The device in this paper supports a compositive method for determining SOC. It saves time and labor, and is more accurate. Automation is the undeniable trend of future analytical chemistry.

Table 4 Data of method determination limitation

Analysis times	Measured value (mg)
1	0.389
2	0.278
3	0.333
4	0.556
5	0.333
6	0.444
7	0.333
SD	0.093
MDL	0.293

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