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## Protocol Article

# Protocol of conjugate evaluation of the biological activity of soils in terms of cellulolytic activity and biological consumption of oxygen



Pavel P. Krechetov, Anna V. Sharapova, Ivan N. Semenkov\*,  
Tatiana V. Koroleva

Lomonosov Moscow State University, Russia

## A B S T R A C T

The article presents protocols for determining the biological activity of kerosene-contaminated soils in terms of two indicators, i.e. cellulolytic activity and biological consumption of oxygen. A method for determining the cellulolytic activity of soils is based on measuring the rate of cellulose decomposition *in situ*. Model test objects (linen fragments 10 × 20 cm weighing 4–6 g) were put in the root layer of soil. A month later, the linen was removed from soil and its weight loss was measured. Cellulolytic activity was estimated by the weight loss of readily hydrolysable organic matter (RHOM) per day (mg/g RHOM per day). The method for determining the biological consumption of oxygen of water was adapted for soils. The indicator characterizes the ability of microorganisms to oxidize organic substances using oxygen for 5 days. The analytic procedure includes taking a soil sample, preparing the suspension (the ratio of soil to distilled water is at least 1:10) and after 5 days measuring the concentration of unspent dissolved oxygen using the oxygen meter. The proposed methods give reproducible and reliable results on the biochemical activity of soil microorganisms in a wide range of soils, e.g. Retisols, Arenosols and Histosols, including those under hydrocarbon pollution.

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## A R T I C L E I N F O

*Method name:* Evaluation of the biological activity of soils in terms of cellulolytic activity and biological consumption of oxygen

*Keywords:* Biological soil health, One health approach, Microbial decomposers, Soil quality, Biodegradation, Organic Matter, Controlled Study, Environmental Factor, Microbial Activity, Ecological indicators

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*Abbreviations:* RHOM, readily hydrolysable organic matter; TBI, Tea Bag Index; BCO, biological consumption of oxygen.

\* Corresponding author.

*E-mail address:* [semenkov@geogr.msu.ru](mailto:semenkov@geogr.msu.ru) (I.N. Semenkov).

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## Specifications table

Subject Area	Environmental Science
More specific subject area	Soil biology
Protocol name	Evaluation of the biological activity of soils in terms of cellulolytic activity and biological consumption of oxygen
Reagents/tools	Distilled water Linen Thermal oxygen meter
Experimental design	The article presents a conjugate method for determining the biological activity of soils by two indicators, i.e. cellulolytic activity and biological consumption of oxygen. The cellulolytic activity is estimated by the weight loss of linen fabric per unit time <i>in situ</i> . Biological consumption of oxygen is measured after five-day airtight oxygen-eliminating incubation of an aqueous suspension of soils.
Trial registration	N/a
Ethics	N/a
Value of the Protocol	The proposed methods give reproducible and reliable results on the biochemical activity of soil microorganisms in a wide range of soils, including jet-fuel contaminated ones. We offer inexpensive, easy-to-prepare and affordable procedures for determining the biological activity of soils that do not require sophisticated equipment and highly qualified personnel. The method for determining the biological consumption of oxygen of water is adapted for soils.

## Background information

The biological activity of soils can be measured both directly through the quantity of organisms of a particular group or their biomass, and indirectly through the rate of organic matter decomposition, O<sub>2</sub> uptake or CO<sub>2</sub> and heat production by a soil microbial community.

Soil organic matter decomposition is a fundamental ecosystem process that allows for the cycling of nutrients. The rate of organic matter decomposition is measured using various test objects, such as filter paper [1–4], linen [5,6] or cotton [7–9] fabric, tea bags [9–16], components of woody or herbaceous plants (needles, leaves and branches) [11,17–19]. Recently, the tea bags have become the most widely used among them with the subsequent calculation of a Tea Bag Index (TBI) [10,20–22] proposed by [23]. The use of standardized material makes it possible to evaluate the contribution of external factors, regardless of the properties of decomposed test objects.

In the article, we propose to estimate the intensity of organic matter decomposition in soils using linen fabric, i.e. a material found in nature.

The most popular methods for assessing the biological activity of soils are those related to the uptake of O<sub>2</sub>. Their main disadvantage is the need for special equipment and highly qualified personnel [24]. We offer inexpensive, easy-to-prepare and affordable procedures for determining the biological consumption of oxygen in soils that do not require sophisticated equipment and highly qualified personnel.

### (1) Procedures of cellulolytic activity measurement

Measurement of cellulolytic activity (Fig. 1) is based on evaluating the rate of linen cellulose decomposition in the most biologically active soil layer (0–10 cm).

### Preparing test objects

The linen fabric must be cut into fragments 10 × 20 cm. The prepared linen fabric is sterilized by ironing at 120–130°C and then kept in an oven at 60°C for 4–6 h to bring it to a constant weight. Then the fabric is weighted on an analytical balance with an accuracy of 0.0001 g. The fragments of fabric are then placed in a fiberglass mesh with a 5 mm or less cell. Fixing linen in the mesh makes

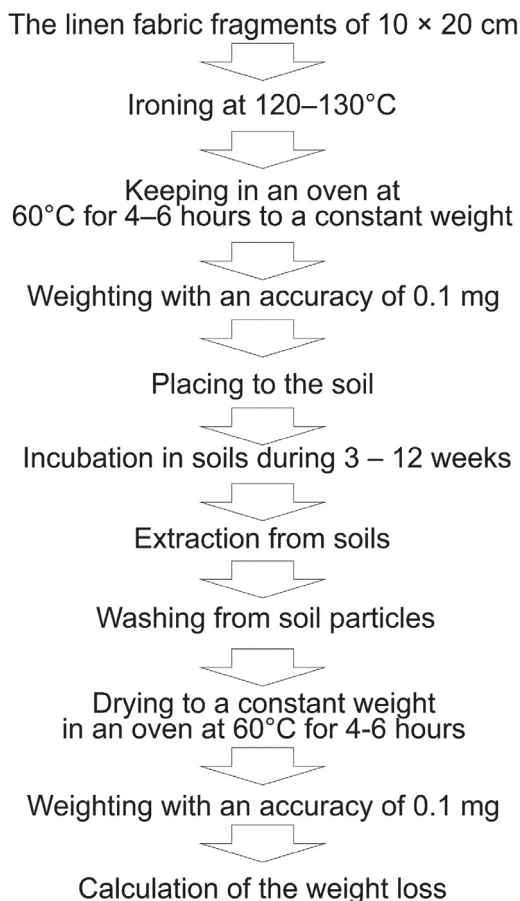


Fig. 1. A workflow of cellulolytic activity estimation in the soil.

the extraction of the test object from soil easier and minimizes its loss if the biological activity is highly intense.

#### *Organization of field observations over the rate of cellulose decomposition*

The plot for studying the cellulolytic activity should be at least  $50 \times 50$  cm. It is recommended to repeat observations at least five times and locate test objects at a distance of no less than 10 cm from each other.

Test objects are placed in the upper 10 cm topsoil layer by cutting to a depth of 25 cm with a bayonet shovel. The first test object is placed in the opening. The previous opening is closed when the next one is made. After placing all test objects, the soil is compacted, if necessary, to ensure close contact with the linen fabric.

The maximum duration of the test linen incubation depends on the biological activity of soil. For example, during peak biological activity in Retisols, Luvisols and Chernozems the incubation period should not exceed 4 weeks (optimally 3 weeks). The incubation period for biologically less active soils could be extended up to 3 months or even the entire growing season [5].

### Extraction and processing of linen residues

Linen fragments removed from the soil are washed from soil particles in the grid to prevent the mechanical loss of decomposed fragments. The washed fragments are dried to an air-dry state and brought to a constant weight in an oven at 60°C for 4–6 h.

### Data processing

The value of cellulolytic activity (A) of soils is calculated by Eq. (1) according to the weight loss of test objects during incubation and is expressed in mg/g of readily hydrolysable organic matter (RHOM) per day:

$$A = \frac{m_1 - m_2}{m_1 \times t} \quad (1)$$

where  $m_1$  and  $m_2$  are the weights of a test object before and after incubation, mg;  $t$  is the duration of incubation, days.

### (2) Procedures of measurements of the biological consumption of oxygen

The method is based on determining the amount of oxygen consumed by soil microorganisms during five-day incubation period for the oxidation of organic compounds in soil suspension kept in airtight containers (Fig. 2). The characteristic value is measured in fresh uncrushed soil samples taken no more than a day ago. If prompt analysis is not possible, the sample may be stored at -20°C.

The procedure of determining the BCO index includes three phases: preparation of suspension, five-day incubation, and measurement of oxygen concentration using an oxygen sensor and a thermal oxygen meter.

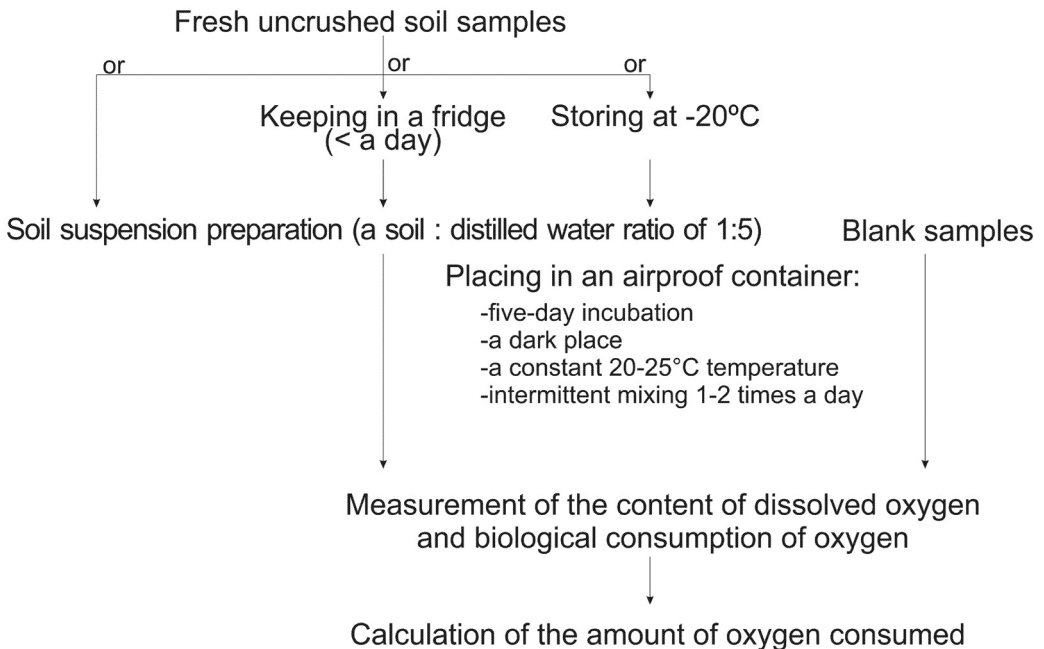


Fig. 2. A workflow of estimation of biological consumption of oxygen in the soil.

### *Preparation of suspension*

To prepare suspension, a sample of soil and distilled water are used. The measurement is repeated at least three times. A sample of soil without roots and other visible inclusions could weight 1 up to 5 g, depending on the content of organic matter and the expected biological activity. The sample of topsoil horizons rich in organic matter should not weight more than 1–2 g. The sample of soils with low content of organic compounds could weight about 5 g.

The samples are placed in the airproof, pre-weighed 50 ml containers. After that, the soil containers are filled to the top with distilled water as to squeeze out the remaining air when the lid is screwed tightly. To determine the amount of added water, the containers are weighed with soil and water. The contents of containers are then homogenized using a rotator for 1 h at medium speed. In parallel, soil moisture is measured in remaining soil samples.

### *Incubation of samples*

The samples are incubated for 5 days in a dark place at a constant temperature (20–25°C) with intermittent mixing 1–2 times a day. Simultaneously with the test samples, blank samples are incubated, i.e. the same containers filled with distilled water (without soil) used to prepare the suspension.

### *Measurement of dissolved oxygen*

After five-day incubation, the content of dissolved oxygen and biological consumption of oxygen were monitored using an Expert-001 thermal oxygen meter (Econic Expert, Moscow, Russia) combined with a Clark DKTP-02.4 electrochemical sensor (Econic Expert, Moscow, Russia). The values are measured under constant stirring of suspension at medium speed using a stirrer applied for the homogenization of suspension, and without excessive input of external oxygen. To minimize oxygen exchange during measurements, the sensor was fixed in the container lid and in the process of measurement the container was hermetically closed with a sensor-bearing lid. Thermal oxygen meter readings were recorded when the value stabilized 3 min after the sensor was placed in suspension.

The amount of oxygen consumed for biochemical oxidation in the sample is calculated from the difference between its concentration in a blank sample and the analyzed sample after five days of incubation.

Biological consumption of oxygen (BCO) is expressed in mmol O<sub>2</sub>/100 g of soil, according to Eq. (2):

$$\text{BCO} = \frac{(O_0 - O_s) \times V \times 100}{1000 \times m \times 32} \quad (2)$$

where O<sub>0</sub> and O<sub>s</sub> are average concentrations of oxygen in a series of blank samples and the analyzed sample after five days of incubation, mg O<sub>2</sub> / l; V is the amount of water used to prepare the suspension, ml; m is the weight of the analyzed soil sample, g (absolutely dry weight); 32 is the factor to convert the results into mmol/100 g.

### **Final remarks**

The proposed method gives reproducible and reliable results on the biochemical activity of soil microorganisms in a wide range of soils, e.g. Retisols, Arenosols, Chernozems, Histosols, Regosols, Technosols [5,25], including those under hydrocarbon pollution [6,26,27].

### **Declaration of Competing Interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. potential competing interests:

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