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THE MACROCALORIMETRIC METHOD IN HYDROBIOLOGY: DESCRIPTION OF THE PILOT DEVICE

A pilot model of differencial-flow macrocalorimeter was made in the Institute of the Biology of the Southern Seas (IBSS), which allows to measure biogenic heat flows several days in a row. This device allows registering temperature changes in vessels and biological objects and transferring this data to the computer. The simultaneous usage of two sensors (in the vessel and in the biological object) gives a better interpretation of the data received. The description of the device, its test results and the possibilities of its utilization are presented in this report.

At present the energy balance approach is one of the basic approaches in hydrobiology. The assessment of the energy metabolism of the hydrobionts (heat dissipation) is the key moment of this approach. The measurement of the oxygen consumption is the index of the energy metabolism for aerobic biological processes and is used in various areas of biology, inclusive hydrobiology. Respirometry, however, can not be used to measure anaerobic hydrobiont metabolic expenditure. In the systems with both aerobic and anaerobic processes (ex. aerobic organisms under hypoxia, bottom sediments, algae mats etc.) repirometry gives inadequate assessment of energy flows. For example, the respirometry results of energy expenditure in the research with bivalve mollusks were considerably understated [2, 3, 6]. The metabolic expenditure in the research with oysters *Crassostrea virginica* was 2 – 3 times less compared to its calorimetric results [2].

Calorimeters, mostly microcalorimeters have been successfully used in hydrobiology for a long time. They allow assessing the small amounts of the heat emission of the smallest organisms and cell populations. When applying microcalorimetery approach to macro objects, the latter should be disintegrated and homogenized and this does not allow applying these results to the entire system. It was shown that the data of the energy expenditure of the whole organism is smaller than the sum of the data of all separately measured tissues [5]. It is possible that the metabolism of the structured mat and not mixed bottom sediments will differ from the metabolism of their homogenat.

Macrocalorimeters used before [2] were just heat insulated vessels with quartz thermometers inside. Such construction did not allow to obtain continual data and to process it with the help of the computer. Due to the shortcomings of the existing macrocalorimeters, the researchers still have to use microcalorimeters with the vessels of 5-20 ml (which is not adequate to the size of animals) in order to study heat production of macroorganisms (mollusks) [4].

A pilot model of differencial-flow macrocalorimeter was made in the Department of Animal Physiology and Biochemistry of the Institute of the Biology of the Southern Seas (IBSS), which allows to measure biogenic heat flows several days in a row. This device allows registering temperature changes in vessels and biological objects and transferring this data to the computer. The simultaneous usage of two sensors (in the vessel and in the biological object) gives a better interpretation of the data received.

The description of the device, its test results and the possibilities of its utilization are presented in this report. Fig.1 shows the device itself. Metrical thermostatic unit includes two Dewar's vessels – the experimental one and the control one (each V-458 ml) with miniature (1,5-2mm) temperature sensors (type HELL -7000), which enable to get temperature readings directly from the object or from the water in the vessel with 0,0001℃ accuracy. If mollusk is the object of the research, the sensor is placed inside the valve, if algae mats are under research the sensor is placed inside the mat. The information from the sensors about the temperature changes in the experimental and testing vessels is received by the electronic unit of the signal converter in order to be processed by the computer later: data smoothing, temperature gain filtration, heat production (mkV/hrs) graphical plotting. The sensors were calibrated in three steps:

1. Assessment of heat production in the vessel without the object (control);
2. Assessment of heat production with the object (experiment);
3. Assessment of heat production with the heater imitator. In this case the resistor with standardized current, equivalent to the heat production of the research object (Δt° - gain, power – up to 0,174 vatt) was used.

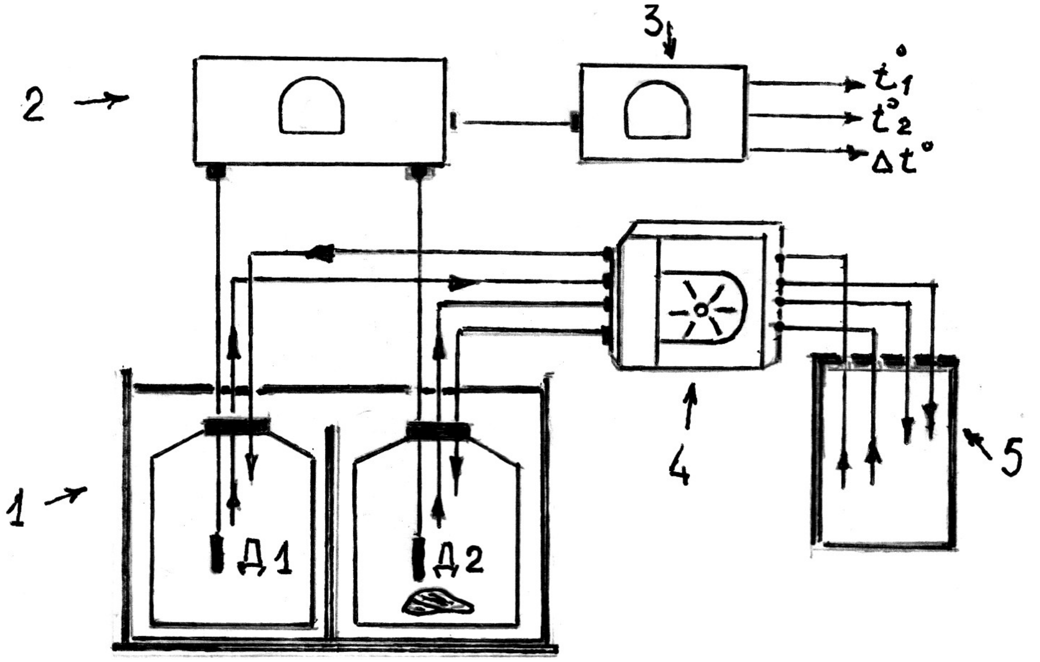

Fig. 1 The differential twin-flow macrocalorimeter: 1. Termostatic reservoir with two temperature sensors (D-1—control, D-2—experimental). 2. Analogue digital converter. 3. Computer. 4. Peristaltic pump. 5. Vessel with makeup water.
Рисунок 1. Схема дифференциального проточного макрокалориметра: 1. Термостатируемый блок для размещения экспериментальных сосудов (Д1- контроль, Д2- опыт). 2. Электронный цифровой преобразователь.3. Дисплей компьютера.4. Перистальтический насос 4х-канальный.5. Подпиточная термостатированная ёмкость с оксигенированной водой.

In long-term working conditions calorimetric system is achieved by the water flow with the help of peristaltic pump (type PP-2-15 Poland), its productivity is 250 ml/hr for the experimental and control vessels. It allows to conduct long-term experiments and use the vessels for respirometrical measurements also, and to assess aerobic metabolism.

This system was tested with oysters of different sizes *Crassostrea gigas*. Two temperature sensors were used: one was placed in the water of the experimental vessel; another was placed inside the oyster through the drilled hole. Between the data of these two sensors there was a stable difference in temperatures of approximately 0,4℃. Measured oyster heat production indexes varied within the limits, which had been measured for oysters in previous research [2, 3]. During the experiments we noticed the variations in the temperature gain (heat production), which are probably caused as well as oxygen consumption [1, 6] by the rhythm of opening and closing of the valves. The details of the experiments will be presented in a separate article. In this report we would like to mention that these experiments demonstrated the effective utilization of the device in hydrobiological research.

The utilization of this device would enable to solve some of aquatic ecology problems:

1. Study of algae mats and algae bacterial biofilms, which involves aerobic and anaerobic processes and where the primary product is created as a result of oxygenic and anoxygenic photosynthesis.
2. Bottom sediments general assessment, where anaerobic processes, such as reduction of sulfate, play an important part as well as aerobic ones.
3. Integral assessment of general metabolism of aerobic microorganisms, e.g. mollusks in the conditions of hypoxia and anoxia, when anaerobic metabolism is used.

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**МАКРОКАЛОРИМЕТРИЧНИЙ МЕТОД В ГІДРОБІОЛОГІЇ:
ОПИС ПІЛОТНОЇ МОДЕЛІ ПРИЛАДУ І ЇЇ МОЖЛИВОСТЕЙ**

Резюме

У Інституті біології південних морів НАНУ створений дослідний зразок диференціально-проточного макрокалориметра, що дозволяє прямо вимірювати біогенні теплові потоки в багатодобових експериментах, що діє. Розроблений прилад дозволяє в режимі реального часу знімати і передавати на комп'ютер інформацію про зміни температури в судинах і біологічному об'єкті. Використовувані електроди можуть поміщатися як у воду, так і всередину біологічного об'єкту. Використання одночасне двох електродів дозволяє оцінювати різницю температур усередині об'єкту і в експериментальній судині, що розширює можливості інтерпретації отримуваних даних. Представлено опис установки, її використання, можливостям застосування.

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**МАКРОКАЛОРИМЕТРИЧЕСКИЙ МЕТОД В ГИДРОБИОЛОГИИ:
ОПИСАНИЕ ПИЛОТНОЙ МОДЕЛИ ПРИБОРА И ЕЁ ВОЗМОЖНОСТЕЙ**

Резюме

В Институте биологии южных морей НАНУ создан действующий опытный образец дифференциально-проточного макрокалориметра, позволяющего прямо измерять биогенные тепловые потоки в многосуточных экспериментах. Разработанный прибор позволяет в режиме реального времени снимать и передавать на компьютер информацию об изменениях температуры в сосудах и биологическом объекте. Используемые электроды могут помещаться как в воду, так и внутрь биологического объекта. Использование одновременно двух электродов позволяет оценивать разность температур внутри объекта и в экспериментальном сосуде, что расширяет возможности интерпретации получаемых данных. Описаны установка, её использование, возможности применения.