PROTOCOL FOR SOIL RESPIRATION MEASUREMENT BY RABIT

Kuzyakov Lab

1	30.09.2016	Aliia Gilmullina		E. Blagodatskaya	Yakov Kuzyakov
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Introduction

Impedance is the resistance to flow of an alternating current through a conducting material. Impedance technology is widely used in microbiology to assess microbial metabolism using either direct or indirect measurements. ***The Rapid Automated Bacterial Impedance technique (RABIT)*** system is now modified for soil measurements.

1. ***The direct method*** is based on the monitoring of microbial metabolites, which can affect the impedance of a culture medium by releasing ionized molecules. Uncharged or weakly-charged substances such as polysaccharides, fats and proteins are metabolized by micro-organisms into highly charged end-products, such as organic acids, fatty acids and amino acids. The resultant changes in the electrical properties (conductivity and resistance) of the culture medium can be measured by metal electrodes placed in the container of the inoculated growth medium.
2. ***The indirect impedance technique*** monitors the electrical conductivity change of alkaline solution due to ionization of CO_2 to carbonate. In this case, the electrodes are immersed into a CO_2 absorbing trap containing alkali, rather than the microbial cultures associated with the direct method. CO_2 produced from the soil microbial community is trapped in an alkaline absorbent solution and causes a decrease in the conductance of the alkaline solution.

The PC and 32 channel unit contain individual short term battery backup systems. These will maintain the system operative (apart from the module heaters) for up to 15 min if the mains fails or is inadvertently disconnected.

Individual incubator modules can be run at different temperatures from 4°C above ambient to a maximum of 50°C. It is recommended to set temperature 2-3°C above ambient temperature.

Important: Sensitivity of device is 1000 – 32767 µS. If cell is empty or contains liquid with conductivity lower than 1000 µS, the system will not detect the cell.

The aim of the method is to measure soil (basal/substrate-induced) respiration by indirect impedancemetry.

The main steps of substrate-induced and basal respiration measurements by RABIT:

1. Prepare the RABIT cells and leave them to stabilize overnight
2. Before starting an experiment choose parameters of incubation and measurement, save your project.
3. About 2 hours before starting experiment (substrate addition) insert the cells to the module to adjust temperature equilibrium and start your test (more information – ***3.d***).

4. Prepare soil samples. Before adding the substrate to soil samples wait for the last measurement of the empty ells in RABIT system. When the last measurement is done, add substrate to soil and insert epp tube with soil sample to the cell.

Important: Do not use values measured when the cells were without soil sample. The first value of analysis is the last measurement done before inserting the samples.

Insert samples at least before 5-10 min left to the next measurement, because samples should be warmed.

Preparation of RABIT cells for indirect method

1. Prepare the base with concentration you need.

Table 1. Conductivity values for different concentrations of NaOH and KOH.

Alkali concentration, M	V of alkali, ml	Initial conductivity value, µS	Amount of C-CO2 can be absorbed until 50% saturation, mg in cell	C-CO2 concentration 50% saturation, mg/l	Conductivity value of 50% saturation, µS
0,05	1,5	~14000	0,225	150	~10000
	3		0,45	150	
0,1	1,5	~26000	0,45	300	~18000
	3		0,9	300	

2. Dispense ≥1.5 ml (≤4 ml) of the NaOH to the bottom of each clean and dry RABIT cell, taking care to minimize the quantity of base drops on the walls of the cell
3. Immediately close the cells as tightly as possible using the rubber caps.
4. Leave cells to stabilize for a minimum of 2-3 hours (preferably overnight) prior to use.

Preparation of soil

1. Cut the Eppendorf tubes (V=2 ml)
2. Weigh 0.5-1 g of soil
3. Pre-incubate soil samples if it is necessary.

Working with software Rapid Automated Bacterial Impedance technique (RABIT)

Operating instructions

1. Starting the program

a. Turn on the PC

b. Turn on the incubator (32 channel unit) by plugging (it doesn't have any buttons for on/off)

c. Run RABIT for Windows

2. Starting a project for choosing incubation parameters

a. Select "test codes" in menu bar

b. Fill in the parameters:

- Test duration (maximum=999 h)
- Time resolution
- Temperature (4-50°C)

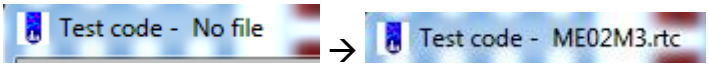
Important: It takes more than an hour to achieve a stable temperature (but if needed temperature is less than previous temperature, it takes **much more time** to cool the incubator). A green lamp at the front of the module will illuminate when temperature is stable.

Use buttons on the module to choose temperature, but module with needed temperature will be chosen automatically by using the code setting.

- Detection criterion (the value must be negative because conductivity will decrease, when this value is achieved it will mean that growth/CO2 production/ conductivity change is detected and the detection point will be shown on the graph by yellow cross)

c. Before closing the window save the project. Select "file" in menu bar → "save as" →the name must be in the format UUNNXX.rtc, where U is an uppercase character, N is a number and X is any character. (ex. TE25T1.rtc)

d. After saving heading of test code window will change:



e. Now the window of test code can be closed.

f. For choosing already prepared test code:

- "test codes" in menu bar → "file" → "open" → choose your project →name of window will change to the name of chosen project → close the window

3. Giving names for the samples and choosing measurement parameters

a. Select "Start" in menu bar. A 

window with a worksheet will

appear.

b. In the top right hand corner of the screen is a chart showing the number of vacant cell locations, running at specific temperatures, ready for accepting a test.

c. In the middle right hand corner of the screen are some user configurable status indication boxes

- "Default value" – 30 min, "set extended value" – manual set of value, "use extended value" – after setting extended value they will appear automatically
- "Sample code entry"
- "Manual entry mode" If the sample code entry option is set to "Bar code" these options are not used. If the "Manual" option is selected then the available options are "Enter every code", "Auto increment" – increasing one of series, "Keep code constant" – the last entered name will appear automatically

d. Fill in the table, enter the following data and press the enter key

- W/s No. will be filled in automatically
- Sample code – the name of the sample, 20 digit field
- Test code – the name of test code is used at the moment
- Temp – the value of temperature

Important: the system will choose the appropriate module based on this temperature information.

- Delay – time delay before starting the first measurement

Important: No reading are taken from the cell in the first 30 min of the test as a finite time is required for the test cells to reach thermal stability. It is recommended to choose 1:30 hours of delay to be sure that cells are stabilized. So if delay is 00:00, the first measurement will be done only after 30 min (if delay is 01:30, the first measurement will be done after 2 hours).

e. When the last line of complete entries has been made, press the enter key to place the cursor to the beginning of the next line

f. Saving the worksheet:

Select "file" in menu bar → "save as" →give the name

g. For choosing already prepared worksheet:

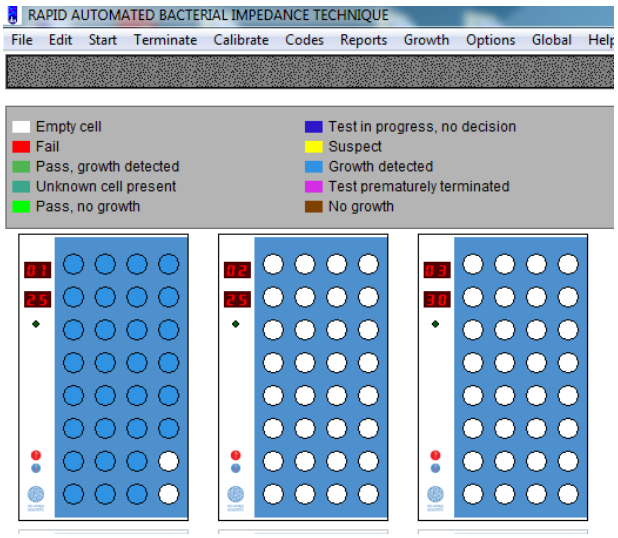
"file" in menu bar → "open" → choose your worksheet)

h. Press "Allocate" to allow RABIT to automatically allocate the cell locations (manual allocation is not possible)

i. When all cells are allocated, press "fit cells" and insert the cells into the module. Drop the cell into socket and push gently until cell bottom in the socket.

j. Cell accepted are indicated by green ticks () appearing in column “Location” on RABIT screen.

k. Press “done”. Now test is running.



4. <u>Preview of results</u>

a. Open the main screen of RABIT. Select the module and click on the cell. Only 2 cells can be previewed simultaneously. The data is updated after every measurement

b. To see the values of conductivity in real time select “Global” in menu task →”Conductivity table”

c. Select “Growth” in menu task. Choose the date (month, day and hour) an experiment have started, protocol used and the cell. Add the cells you want to see on graph (max = 12). On the left hand side the following options can be selected:

- Individual – only one curve on graph

Normal – absolute values

Differential – rate

- Comparative – many curves on graph

Relative – the change of conductivity

Absolute

Autoscale – percentage

5. <u>Test termination</u>

a. Select “Terminate” from the menu bar

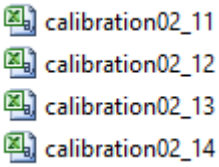
b. Select the module and mark the cells to be terminated by clicking on them. A black border will appear around the cell.

c. Remove the cells. A few seconds after each cell is removed the cell status indicator will change to the following colours. If the test is still in progress the status indicator will change to **purple**, the message on the diagnostic screen will indicate “Premature removal of cell from module XX” and then after a few seconds colour change to **white** (empty cell). If the test had completed, the display will change directly to **white**.

d. After changing the colour and changing the number of terminated cells from 0 to x, press “done” and close the window

e. If test was in progress, diagnostic screen will show an error. When all cells were inserted, click on diagnostic screen and clear the message.

f. All cells locations must be properly terminated, otherwise a new test in an empty cell cannot be started. The cells vacant table (start→ on the top right hand table) will not be updated and the cell positions cannot be allocated for new tests.



6. Data exportation

a. Select "Reports" from the menu bar. Choose the date (month, day and hour) an experiment have started, protocol used and the cell(s). Select "File" from the menu task → "export all", give the name "xxx_11". A format of the data is CSV format. Every cell will have its own file.

b. Exported files can be found on desktop (shortcut for data transfer)

7. Cleaning of RABIT Cells

a. Remove epp tube with soil

b. Clean it by using tap water and then 3 times rinse by distilled water.

c. Dry at 40°C overnight

d. Periodic cleaning of the electrode pins that contact the socket with scouring pad or something similar will ensure good electrical connections. If necessary, detach the tube from the cell base assembly and place the cell base and/or tube in a beaker containing 0.1M HCl and then ultrasonicate for 30 minutes. After sonication, wash the cells/tubes in deionised water, dry the cells and then re-attach the tube to the cell.

8. Problems and decisions

a. There is no green sticks after putting the cells into the module

- Check the conductivity through "Conductivity table"(4.b), if the value is "0", the cell isn't on the bottom of the socket, push it gently until it is on the bottom. If value is about 1000, prepare more concentrated base solution.

b. During exportation a message "An attempt to access an invalid address was made" appears.

- Close the program, restart the system and try to export again

9. Calibration

a. Conductivity is very sensitive to temperature, thus, if temperature of incubation is changed, new calibration is necessary.

b. Prepare solution of Na_2CO_3 and put different volumes of the solution into epp tubes (cut only the cap of tube).

Important: Saturation of alkali cannot be higher than 70%.

Table2. Calibration (Changed by Anna Gunina)

			Reagents in cell:			
Concentration (NaOH), M	0,05	0,05	0,05	0,05	0,05	0,05
Volume (NaOH), ml	1,5	1,5	1,5	1,5	1,5	1,5
			Reagents in epp tube:			
Concentration (Na_2CO_3), M	0,047	0,047	0,047	0,047	0,047	0,047
Volume (Na_2CO_3) in epp, ml	0,05	0,08	0,15	0,18	0,2	0,4
Amount(Na_2CO_3) in epp, ug	249.1	398.6	747.3	896.8	996.4	1992.8
Amount(CO_2) in cell, ug	103.4	165.4	310.2	372.2	413.6	827.2
			Addition of acid			
Concentration (H_2SO_4), M	1	1	1	1	1	1
Volume (H_2SO_4), ml	0,5	0,5	0,5	0,5	0,5	0,5
				Products:		
Amount (C-CO_2) in cell, ug	28.2	45.1	84.6	101.5	112.6	225.6
Concentration (C-CO_2), ug/ml	18.8	30,1	56.4	67.7	75.1	150.4
Saturation of NaOH, %	6,3	10.0	18.8	22.6	25.0	50.0

c. Put epp tubes into the cell, close it by grey rubber caps.

d. Inject 0,5 ml 1M H_2SO_4 into epp tube through closed cell.

Important: Volume of liquid in epp tube should be less than 1 ml.

Calibration graph

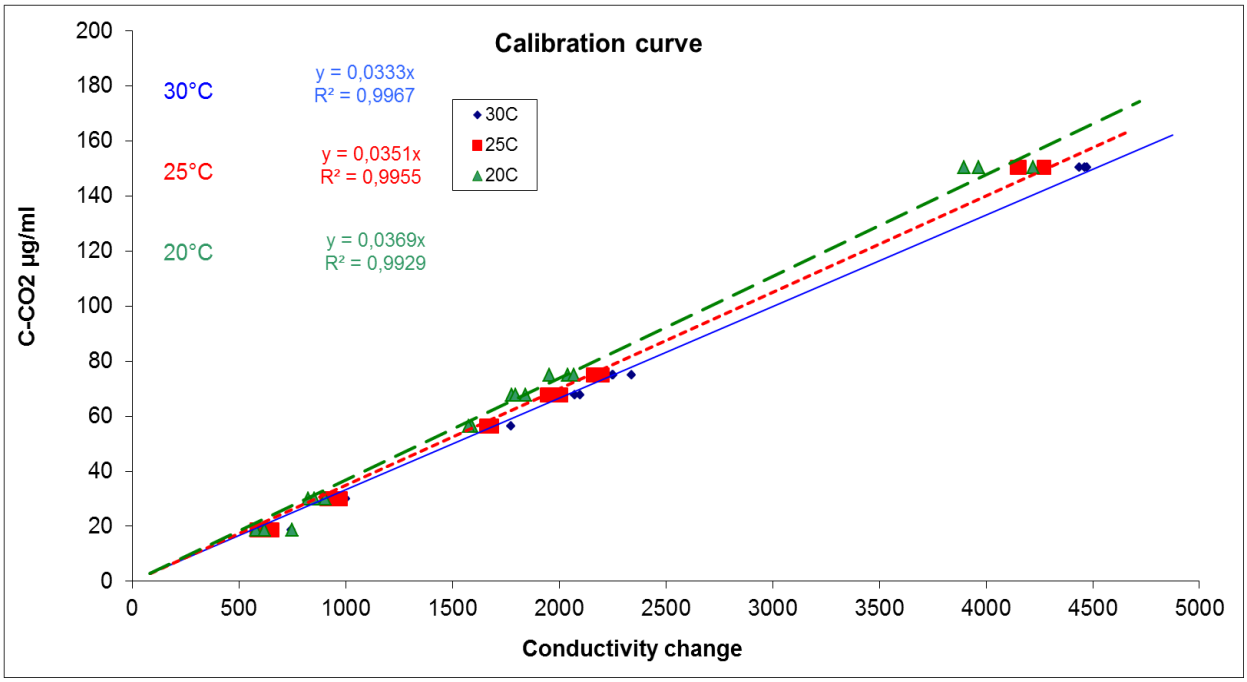


**Figure and measurements produced by Anna Gunina.*

For 25°C

$$C(C-CO_2) = \frac{((0{,}0351 * \Delta EC)) * V}{m};$$

where C(C-CO_2) – C-CO_2 concentration, µg/g;

ΔEC – electrical conductivity change, µS;

V – volume of alkali, ml;

m – weight of dry soil, g.