

Construction of a respirometer for wastewater characterization



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Appendix 1: Respirometer user instructions (in Swedish)

1 Introduction

Respirometry is a useful laboratory technique that in the field of wastewater treatment can be used for e.g. assessment of biodegradability for wastewater characterization (Melcer et al., 2003). Commercial respirometers can be expensive, it can therefore be beneficial if one can build one oneself at a lower cost. For this project, a respirometer is built from relatively inexpensive equipment (total cost < 1 000 Euro). The project is summarized in this report.

2 Theory of respirometry

Respirometry refers to measurement of the respiration rate of microorganisms and is for wastewater treatment processes usually measured through the decrease in electron acceptor, e.g. O_2 for aerobic oxidation or NO_3 for anoxic oxidation, or sometimes the production rate of reaction products, during an experiment (Spanjers & Vanrolleghem, 2016). During the respiration reaction substrate (e.g. readily biodegradable COD) is consumed together with the electron acceptor, generating energy for growth for the microorganism. At the same time reaction end products such as CO_2 are generated. In the case of wastewater treatment, these facts can be utilized to obtain information about the biomass (e.g. nitrification rate) and wastewater sample (e.g. amount of substrate).

In the case of batch experiments with a wastewater sample in a completely mixed reactor, the mass balance equation for the oxygen consumption can be written as Equation 1:

$$\frac{dS_{O_2}}{dt} = kLa * (S_{O_2,sat} - S_{O_2}) - r_{O_2} \quad (1)$$

where S_{O_2} : oxygen concentration [$mg.L^{-1}$];

t : time [s];

kLa : oxygen transfer coefficient [s^{-1}];

$S_{O_2,sat}$: oxygen concentration at saturation [$mg.L^{-1}$];

r_{O_2} : oxygen utilization rate [$mg.L^{-1}.s^{-1}$].

If the aeration is switched off and the water surface is covered to prevent oxygen intrusion, the mass balance equation simplifies to Equation 2:

$$\frac{dS_{O_2}}{dt} = -r_{O_2} \quad (2)$$

Under non limiting conditions (neither substrate nor DO), the oxygen utilization rate (OUR) and thus the measured DO decline is linear. Therefore, by discretizing Equation 2 according to Equation 3, the oxygen utilization rate (OUR) can be calculated directly from the slope of the measured DO concentration.

$$-\frac{\Delta S_{O_2}}{\Delta t} = r_{O_2} \quad (3)$$

When the OUR is known, the amount of consumed oxygen can be calculated from Equation 4:

$$\Delta S_{O_2} = -r_{O_2} * \Delta t \quad (4)$$

An example setup of a respirometer intended for use with wastewater applications is shown in Figure 1. The setup includes: a laboratory scale reactor located in a water bath for temperature control; a DO probe connected to a data logger and aeration controller; aeration device connected to aeration controller; mixing device; and a cover for the liquid film to avoid oxygen intrusion during non-aerated periods. This type of respirometer is used in batch mode, meaning that no continuous flow through of wastewater is possible.

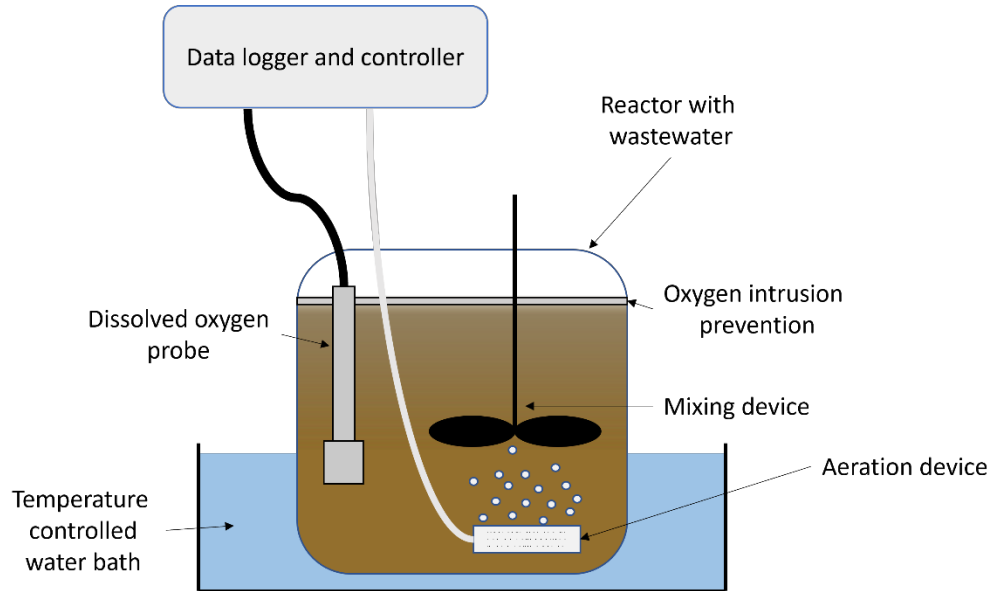


Figure 1. Example of experimental setup for a respirometer.

When the respirometer in Figure 1 is used for wastewater characterization, several methods exist. Ekama et al. (1986) presented a batch test where mixed liquor activated sludge is added to a wastewater sample and the OUR is logged. During this test, the OUR is high initially when readily biodegradable substrate is consumed. When all readily biodegradable material is consumed, a sudden drop in OUR is noticed as the respiration is halted due to the hydrolysis required to make more complex organic material available for the biomass. By calculating the area below the OUR curve and subtracting the OUR originating from slowly biodegradable substrate, the oxygen used for degrading the readily biodegradable COD can be estimated. From this, the readily biodegradable COD (S_B) can be calculated through Equation 5 (Ekama et al., 1986):

$$S_B = \frac{1}{(1 - Y_{OHO})} * \Delta S_{O_2} * \frac{V_{ML} + V_{WW}}{V_{WW}} \quad (5)$$

where S_B : readily biodegradable COD [mg COD.L⁻¹];

Y_{OHO} : yield of ordinary heterotrophic organisms [mg COD.mg COD⁻¹]

V_{ML} : volume of added mixed liquor [L];

V_{WW} : volume of added wastewater [L].

An example of the use of this method with an industrial wastewater in Sweden is shown in Figure 2. Sometimes more than two biodegradable COD fractions can be required to accurately model the reactions, e.g. for industrial wastewaters. This could be required for the sample in Figure 2, where an initial plateau can be recognized around 1.75-2.3 h before the OUR settles on about 8 mg.L⁻¹.h⁻¹.

Another respirometric method was developed by Wentzel et al. (1995) to estimate the initial concentration of ordinary heterotrophic organisms in the wastewater. In this batch test, raw wastewater is used without addition of mixed liquor activated sludge, meaning that the measured OUR is dependent on the initial biomass concentration and subsequent growth as S_B is consumed. Due to the exponential growth of the

biomass, the initial concentration in the sample can be calculated. An example of the results from such a test for wastewater from a plant in Sweden, using the respirometer built according to this report, is shown in Figure 3.

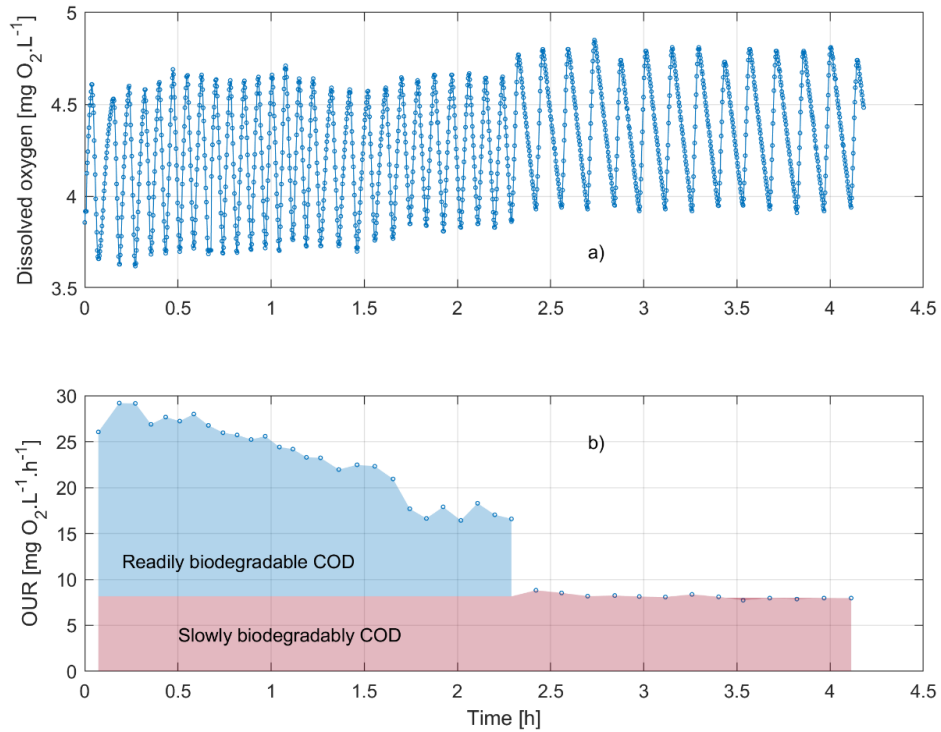


Figure 2. a) Variations in dissolved oxygen concentration and b) OUR curve with division of COD into two fractions during respirometry test according to Ekama et al. (1986).

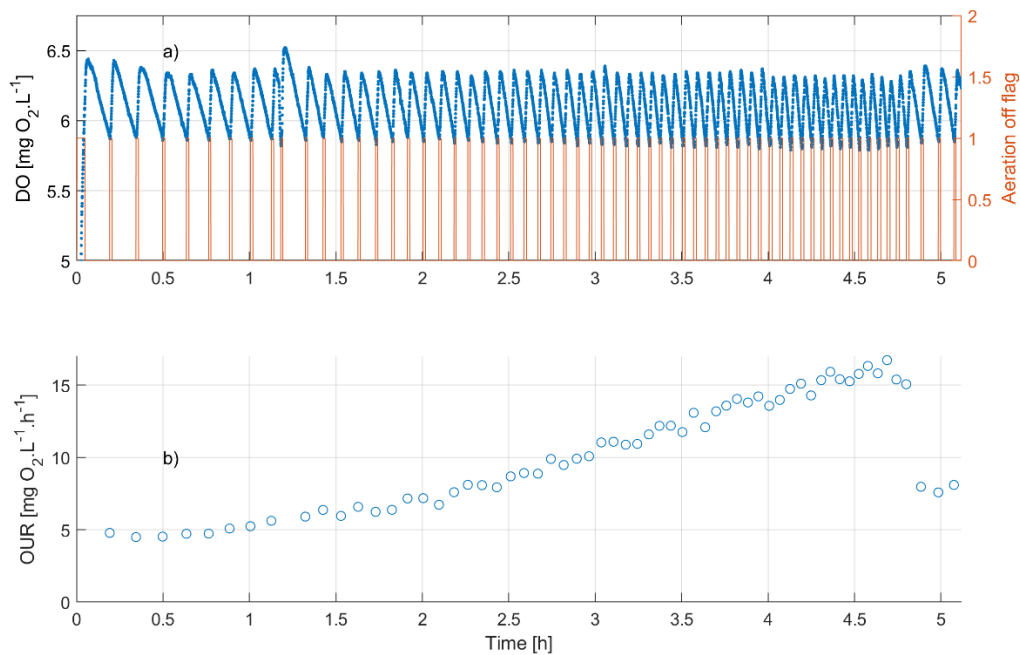


Figure 3. a) Variations in dissolved oxygen concentration and b) calculated OUR during respirometry test according to Wentzel et al. (1995).

Vanrolleghem et al. (1999) mention several other biological model constants which can also be estimated from respirometry, e.g.:

- Yield coefficient of ordinary heterotrophic organisms (OHO), Y_{OHO} ;
- Yield coefficient of autotrophic nitrifying organisms (ANO), Y_{ANO} ;
- Maximum specific growth rate and decay coefficient of OHOs, μ_{OHO} and b_{OHO} ;
- Half-saturation constant for S_B for OHOs, K_S ;
- Maximum specific growth rate and decay coefficient of ANOs, μ_{ANO} and b_{ANO} .

3 Construction

3.1 Components

The base for the respirometer is a Raspberry Pi 3B+ computer running the Raspbian OS (v. 3.6) while the sensors are from Atlas Scientific (<https://atlas-scientific.com/>). The case for the respirometer is built from 15 mm plywood (top and bottom) as well as 6 mm plywood (walls). The complete list of components is listed in Table 1.

Table 1. List of components required for respirometer construction.

Component	Brand	Model	#
Microcomputer	Raspberry Pi	3B+	1
Power supply	Raspberry Pi	Official RP3/3B+ PSU 2.5 A	1
Touch screen	Raspberry Pi	RP3 7" touchscreen display	1
Screen cable	Raspberry Pi	Flex cable for Raspberry Pi camera – 300 mm	1
DO sensor	Atlas Scientific	Lab Grade Dissolved Oxygen Probe	1
DO calculation circuit	Atlas Scientific	EZO Dissolved Oxygen Circuit	1
Temperature sensor	Atlas Scientific	PT-1000 Temperature Probe	1
Temperature calculation circuit	Atlas Scientific	EZO RTD Temperature Circuit	1
BNC Extension Cables	Atlas Scientific	112 mm BNC male to BNC male	2
Micro SD-memory card	SanDisk	SDXC 64 GB 120 MB/s UHS-I Class 10	1
Sensor connector	Whitebox Labs	Tentacle T3 for Raspberry Pi	1
BNC-port	N/A	BNC female-female panel mount	2
USB-port	Adafruit	Panel mount USB cable male-male	2
Prototype board	N/A	For soldering components onto	1
Breadboard	N/A	-	1
Smart wall plug	N/A	433 MHz with remote control	1
433 MHz receiver	N/A	-	1
433 MHz transmitter	N/A	-	1
433 MHz antenna	N/A	Spiral antenna for soldering	2
Distance screw	N/A	M2.5 15 mm	8
Nut	N/A	M2.5	6
Jumper cable	N/A	Male-female	3
Jumper cable	N/A	Female-female	2
Extension plug	N/A	With 3 connections	1
Aquarium pump	Tetra	APS 100	1
Aeration stone	Tetra	AS35	
Silicon tube	-	2 m	1

3.2 Assembly

The assembly was done as follows:

1. The Raspbian OS is installed on the micro-SD card through Raspberry Pi imager (v. 1.4) and then installed in the Raspberry Pi.
2. The distance screws and nuts are installed on the Raspberry Pi and prototype boards.
3. The Tentacle T3 (board to hold the EZO circuits and connectors to the sensors) is attached on top of the Raspberry Pi through the GPIO ports (thus solderless).
4. The EZO circuits (converts the raw DO and T signals to mg/L and °C) are changed to operate in I2C mode (required for the Tentacle T3) manually with the help of a breadboard and jumper cables (see instructions through Atlas Scientific).
5. All required libraries are installed on the Raspberry Pi (numpy, scikit-learn, matplotlib) and the Raspberry Pi is configured to work in I2C mode.
6. The EZO circuits are connected (solderless) to the Tentacle T3 at the site of the BNC connectors.
7. The 433 MHz transmitter is soldered to the prototype board with 2-3 antennae soldered in series soldered to the transmitter.
8. 3 jumper wires are soldered on the prototype board (5V, ground and data) and connected to the Raspberry Pi GPIO ports 2 (5V), 6 (GND) and 16 (GPIO24) as well as the transmitter VCC, GND and DATA ports.
9. The flex cable is connected to the Raspberry Pi display connector as well as the connector on the 7-inch touch screen. The GND and 5V connections on the touch screen are connected to the Raspberry Pi GPIO ports 4 (5V) and 9 (GND).
10. The BNC extensions are connected to the BNC ports on the Tentacle T3.
11. The USB cable extensions are connected to the USB-ports.
12. The plywood sides and top are sawed to shape, with a hole in the top sawed and routed to fit the screen.
13. 2 holes are drilled in the front of the case and the BNC panel mount ports are connected through it. The BNC extension cables are connected to the panel mounts on the inside of the box.
14. The smart wall plug as well as the Raspberry Pi power supply are connected in the extension plug. The aquarium pump is connected to the smart wall plug.
15. Finally, the box is assembled and the sensors are connected to the BNC ports on the outside of the case while the wall connector and the 2 USB-extensions are fed through a hole to be accessible on the outside.

The assembled Raspberry Pi with screen and 433 MHz transmitter (without box and sensors attached) is shown in Figure 4.

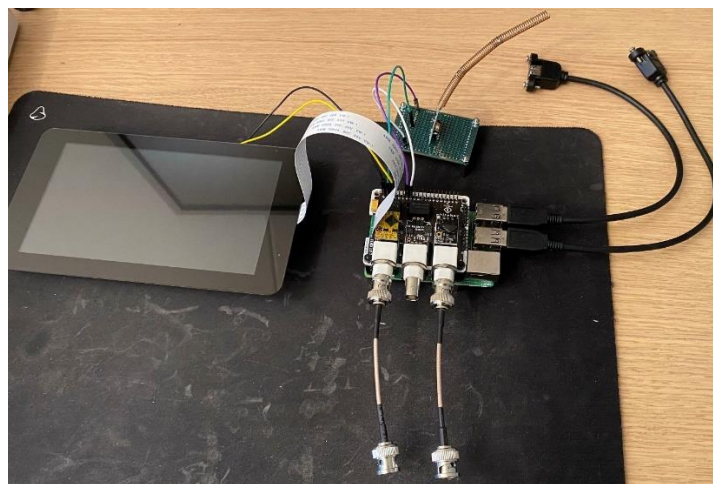


Figure 4. Raspberry Pi assembled (without box and without DO and T sensors connected).

4 Programming

The programming for the project was done in Python 3. The user connects to the Graphical User Interface (GUI), which is used to communicate with the aeration controller and data logger. As new information is collected from the sensors, the information is logged and sent to the view to update the figures and to the aeration controller for control actions. The interactions between the three main parts of the program are showed in Figure 5.

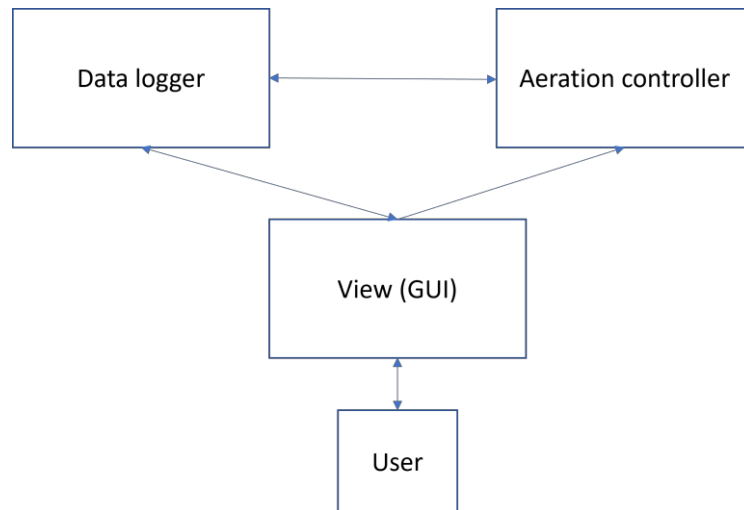


Figure 5. Design of interactions between the view interface, data logger, aeration controller and user.

4.1 Transmitting and receiving signals from the smart wall plug

The smart wall plug is controlled through transmission of 433 MHz radio waves from a remote control, which turn the plug on or off. The signal sent out from the remote control can be intercepted by connecting the 433 MHz receiver to the Raspberry Pi. The signal can then be plotted and analysed. Each button press corresponds to a signal as a sequence of ones and zeros with different intervals between them. The on and off signals were transcribed manually from the plot and the different intervals noted by zooming in on the plot. By writing code to send out the same signal from the transmitter attached to the Raspberry Pi, the outlet can be controlled on or off. This enables control of the aeration without any modification to the high voltage cables.

4.2 Graphical User Interface (GUI)

The GUI for the respirometer was built using Tkinter. After running the main program, the user can choose to calibrate the DO sensor or run a new test. The user enters test name and username, all results are then written to a text file based on the names in real time (thus there will be no data loss if the program crashes). Once an experiment is started, the GUI displays a plot of the DO concentration over time as well as the OUR. A simple dashboard with the current DO and temperature is updated at each sampling time.

4.3 Data logger

The data logger is used to sample new DO and temperature information from the sensors (default sampling time 2 s) as well as write information to a text file during the experiment. The following information is recorded at each sampling time:

- DO [mg/L];
- Temperature [°C];
- Time elapsed [s];

- Date and time;
- Aeration flag (aeration on or off).

4.4 Aeration controller

The aeration controller contains settings for DO thresholds to start and stop aeration as well as codes to turn the smart wall plug (and thus aeration) on or off. It receives input from the data logger on the current DO concentration and sends back the status of the aeration (on or off). At each sampling interval, the aeration controller checks whether the DO is above or below the threshold to start or stop aeration. If it is, a signal is sent out from the transmitter to start or stop aeration. The aeration controller is run on a separate thread from the rest of the respirometer.

5 Test of the respirometer in practice

The respirometer was tested in practice to characterize wastewater from two different treatment plants in southern Sweden. The experiments were mainly performed to obtain the influent concentration of heterotrophic biomass to the treatment plant, according to the method presented by Wentzel et al. (1995). The experiments were performed on raw wastewater with addition of a buffer solution (KH_2PO_4) to maintain stable pH at 7.5 ± 0.1 . The wastewater was added to an E-piston and small pieces of cellular plastic were sprinkled on the surface to avoid oxygen diffusion through the surface. The E-piston was placed on a magnetic stirrer in a tempered water bath (20 °C). The DO sensor was calibrated in water saturated air before each test as the sensor type (galvanic oxygen probe) tends to drift quite quickly. The measured DO was validated with an external portable DO sensor (Hach) at several occasions. The finished setup for the respirometer is shown in Figure 6.

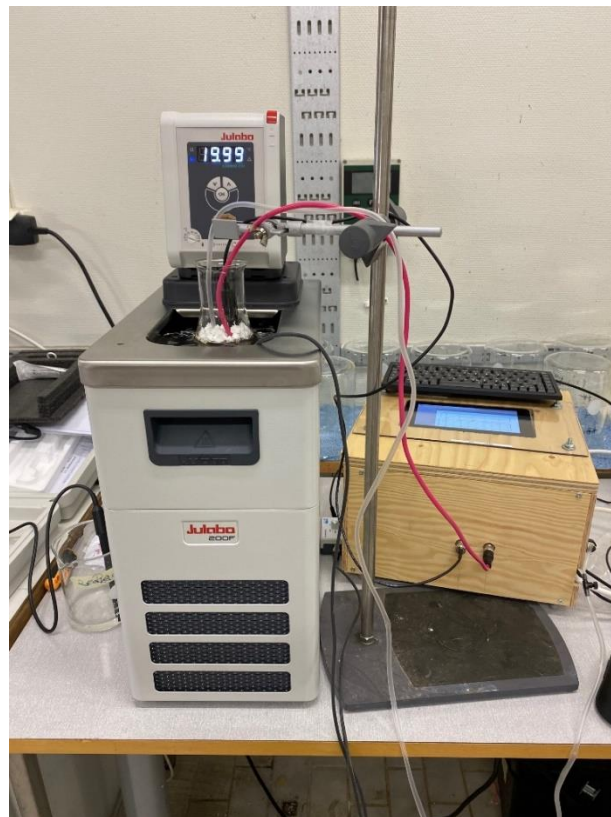


Figure 6. The finished respirometer setup with water bath at a wastewater treatment plant for raw wastewater analysis. An example of data collected with the respirometer during a test is shown in Figure 3 earlier in the report.

6 Lessons learned

A project like this is quite complex when your background is not in electrical engineering, as in my case. As with most complex projects, you must start with a curiosity and the attitude that everything is solvable. Some lessons I have learned through this project:

1. Increased my Python programming skills with:
 - a. GUI-design.
 - b. Real time plotting.
 - c. Real time data sampling from sensors.
 - d. Real time communication between data logging and controller. This requires parallelization and/or scheduling of tasks to work.
 - e. Communication through GPIO on Raspberry Pi.
 - f. Radio transmission communication with Raspberry Pi.
2. Deeper knowledge of different types of oxygen sensors and their pros and cons:
 - a. Galvanic DO sensors have a fast response time but tend to drift and must be calibrated each day of use (YSI, 2009).
 - b. Optical DO sensors have a longer response time (YSI, 2009) and are less suitable for respirometry.
 - c. Water saturated air gives the same voltage output from a galvanic oxygen probe as air saturated water. The galvanic DO sensors can thus be calibrated by putting it in water saturated air and calibrating it.
3. Deeper knowledge of respirometry.

6.1 Challenges

The main challenges during the project were software related, i.e. getting everything to run as intended (and run smoothly without lag). Some of the difficulties encountered and how they were solved:

Lag: the code involves several lines with sleep functions. Since this type of function causes everything to stop until the code continues, this caused severe lag and made the software unusable. Two different remedies were found: 1) schedule some tasks to be repeated after a certain time. There is a function in Python that works similarly to sleep but schedules the code to run again after a certain time (without pausing everything else). This worked well for many tasks, such as checking whether the DO thresholds had been crossed. 2) threading: by separating the controller from the rest in a separate thread, the function of the respirometer was drastically improved.

Real time plotting: initially the real time plotting was built to write a new figure over the old one for each data point. This worked fine for a while, but since the data is updated every 2 seconds the memory ran out eventually. This caused the plot to freeze. Fortunately (since I was running an experiment at the time), the controller runs on a separate thread and continued to read and log values as well as control the aeration. Since I write the data directly to a file as they are read, no data loss occurs if the program crashes. In the end, the plotting was solved by destroying the old plot before each update, freeing up memory. The final version continues to plot data for the several hours that I have run experiments for without freezing.

Weak transmission signal for 433 MHz antenna: sometimes the signal would be too weak to reach the smart wall plug with only one antenna installed. The solution was to solder another antenna to the first antenna (and in some cases, since I have built 3 respirometers in total, 3 antennae had to be soldered together).

Acknowledgements

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Respirometer - användarinstruktioner

1 Inledning

Detta dokument beskriver hur respirometern används vid respirometrieförsök.

2 Delar

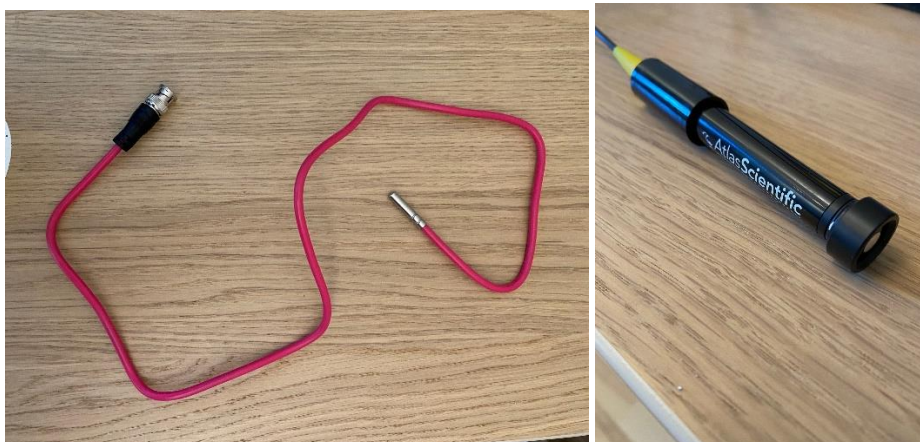
Följande lösa delar krävs för användning av respirometern:

- Respirometerlådan med dator (Raspberry Pi), skärm och anslutningar (Figur 1).
- DO-sensor (Figur 2, t.h.).
- Temperatursensor (Figur 2, t.v.).
- Luftslang.
- Luftsten.
- E-kolv (250-500 mL) med vatten för kalibrering av sensorn.
- Parafilm/tejp.

Utöver detta krävs användning av ett tangentbord och mus (ej trådlösa).



Figur 1. Respirometer i låda.



Figur 2. Temperatursensor (t.v.) och DO-sensor (t.h.) från Atlas Scientific.

3 Användning

3.1 Starta upp datorn

Respirometern startar automatiskt när sladden pluggas i ett eluttag. **VIKTIGT** att inte datorn stängs av genom att rycka ur sladden ur uttaget, detta kan skada datorn och omöjliggöra ytterligare experiment. Se istället avsnitt 3.2 nedan för instruktioner för avstängning.

3.2 Stänga av datorn

Datorn stängs av genom att trycka på hallonsymbolen i övre vänstra hörnet, trycka på ”logga ut” och sedan ”shutdown”. **VIKTIGT** att inte datorn stängs av genom att rycka ur sladden ur uttaget, detta kan skada datorn och omöjliggöra ytterligare experiment.

3.3 Kalibrering av DO-sensor

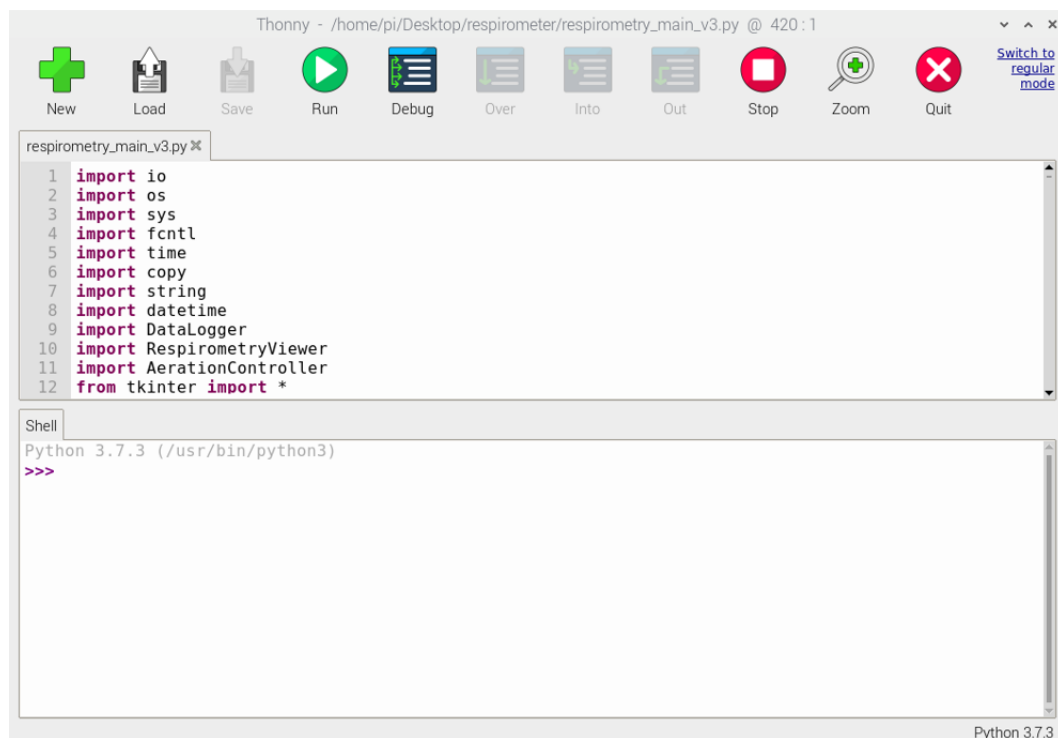
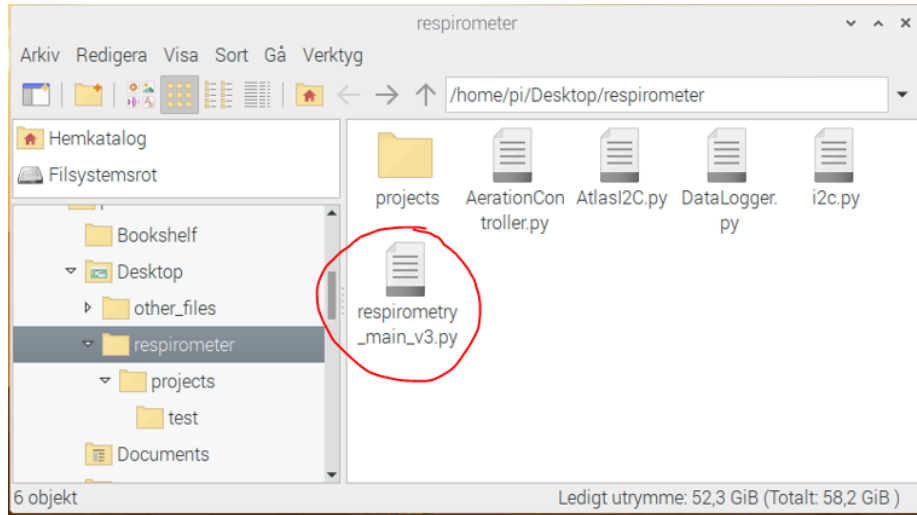
Innan varje respirometrieförsök ska DO-sensorn kalibreras. Detta görs genom att placera sensorn i en atmosfär mättad med vattenånga. Detta åstadkoms genom att:

1. Fyll E-kolv med vatten (ca 20 °C, med andra ord inte jättevarmt och inte jättekallt) till ungefär hälften av volymen.
2. Se till att sensorn är torr, är det våt badda den **försiktigt** med hushållspapper. Membranet är känsligt och om man är för hårdhänt kan det gå sönder.
3. Koppla in sensorn i respirometern och slå på strömmen genom att sätta i stickkontakten i ett uttag.
4. Placera sensorn i E-kolven så att membranet fortfarande är torrt men placerat 1 cm från vattenytan. Råkar man nudda vattnet med sensorn, upprepa punkt 2.
5. Placera parafilt eller tejp över öppningen på E-kolven. Lämna endast ett lite hål (så litet som möjligt) för att fortsatt hålla kontakt med atmosfären. Syftet är att mäta utrymmet ovan vattenytan med vattenånga.

När dessa steg är gjorda, starta programvara för respirometer och kalibrera sensorn enligt stegen nedan.

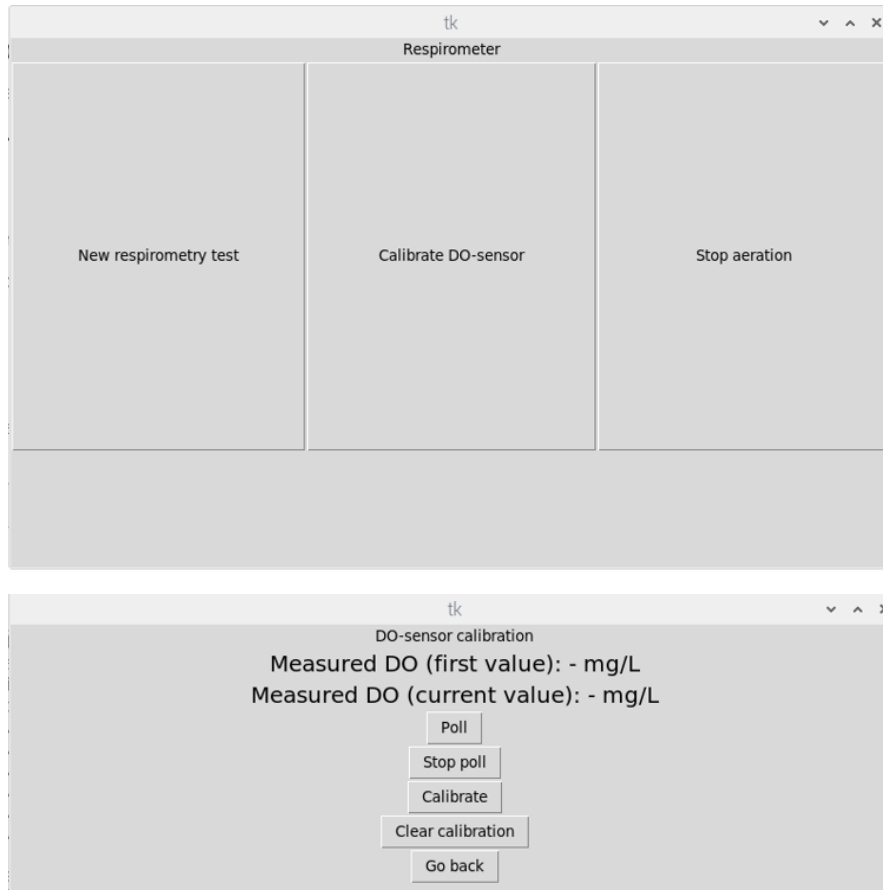
6. Gå till mappen ”respirometer_v4_b” som ligger på skrivbordet, dubbelklicka på mappen.

7. Klicka på filen ”respirometer_main_v4.py”, vilken då öppnas i programmet Thonny.

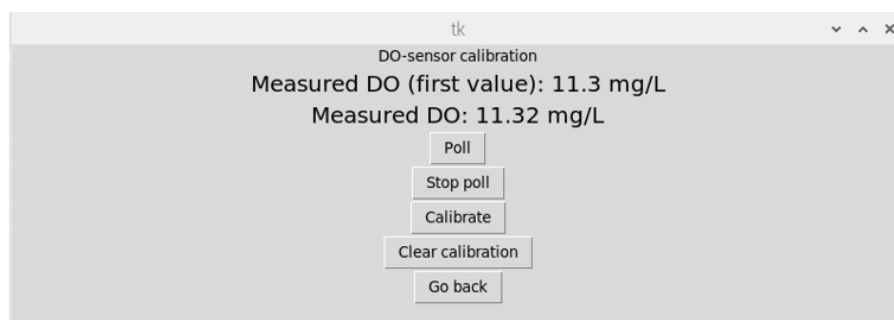


8. Klicka på ”run” för att köra programmet.

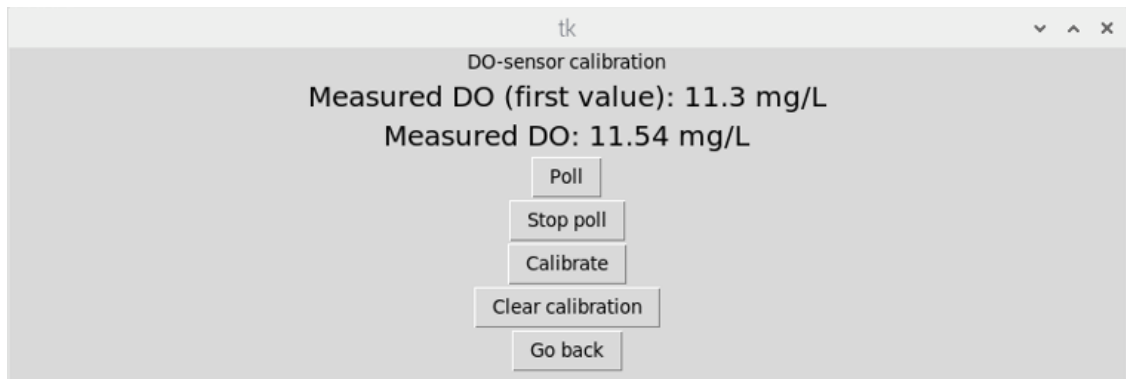
9. När programmet startat, tryck på knappen ”Calibrate DO-sensor” för att komma till kalibreringssidan. Kalibreringssidan visar ännu inga värden för DO, enligt andra figuren nedan.



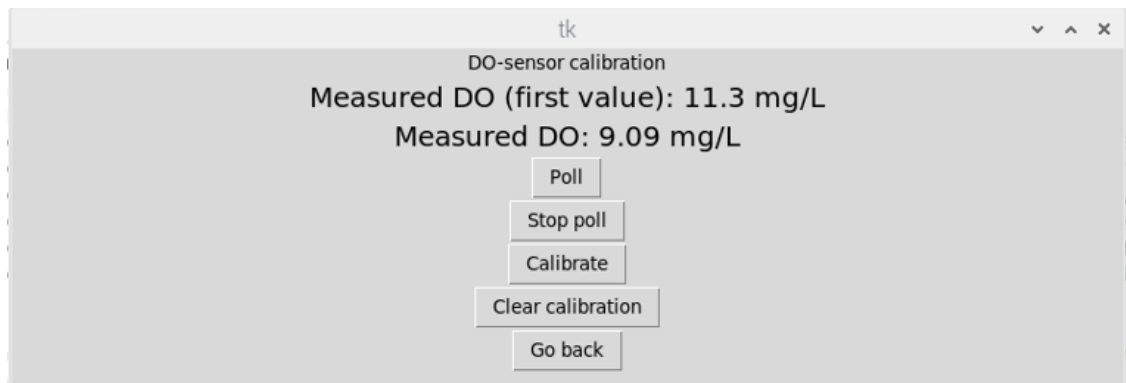
10. Klicka på ”Poll” för att börja samla in och visa värden från sensorn. Värdet uppdateras varannan sekund, under tiden som data samlas in blir den en kort fördröjning vilken gör att programmet kan upplevas lite laggigt. Det första värdet som visas när man startat insamling av data (first value) sparas och visas högst upp. Det senaste värdet visas på den andra raden (current value). Detta för att kunna se förändring över tid. **OBS! Är DO-värdet som läses mycket lågt, nära noll, är det indikation på att elektrolytlösningen i sensorn tagit slut. Kontakta i så fall Christoffer Wärfff för att lösa problemet.**



11. **Låt sensorn sitta i E-kolven i 20 minuter** för att låta luften mättas med vattenånga. Förmodligen kan du se en förändring i uppmätt DO-värden efter denna tid. DO bör efter dessa 20 minuter vara stabilt (små förändringar från mätning till mätning kan dock förekomma).



12. Tryck på knappen "Calibrate". Sensorvärdet kalibreras och bör nu visa 9.09 – 9.10 mg/L. Kalibrering har då lyckats.

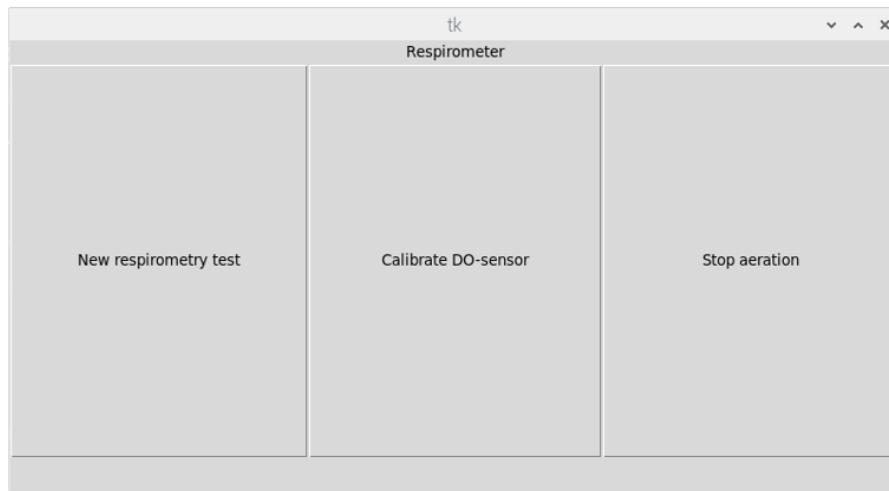


13. Tryck på "Go back" för att komma tillbaka till huvudmenyn.

3.4 Start av respirometritest

OBS! Sensorn ska kalibreras innan varje försök. Följ därför avsnitt 3.3 för kalibrering av DO-sensorn innan start av respirometritest. För att starta testet, följ arbetsgången nedan.

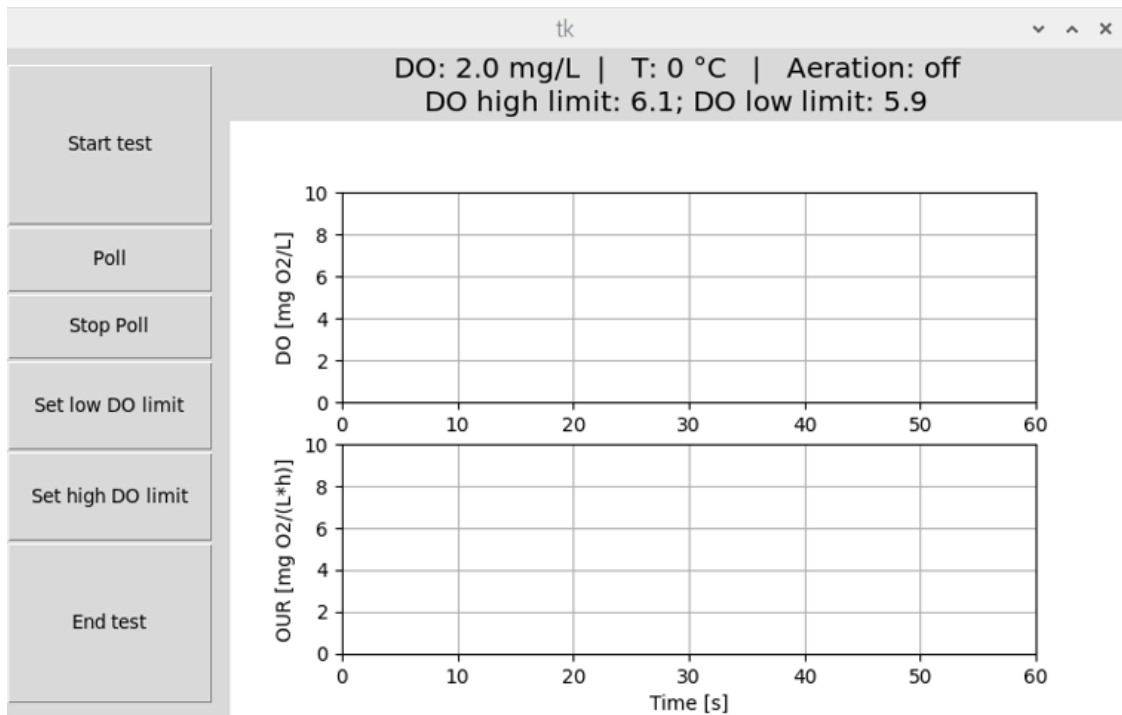
14. Vid huvudmenyn på respirometern, klicka på "New respirometry test".



15. Välj ett lämpligt namn på testet och ange vem som utfört det. Klicka sedan på "Confirm" för att komma till testvyn.
 - a. Lämpligt format på testnamn (använd ej Å, Ä och Ö): ARV_yymmdd_test#.
 - b. Exempel på namn för Öresundsverket: Oresundsverket_211214_1



16. Klicka på "Poll" för att börja mäta aktuell DO-halt (testet är ej startat ännu). Aktuellt DO-värde syns längst upp på skärmen. Gränsvärden för att starta/stoppa luftning kan nu ställas in.
17. Gränsvärde för lågt DO-värde bör vara $> 4,0$ mg/L medan gränsvärde för högt DO-värde bör vara $< 7,5$. Skillnaden mellan högt och lågt värde bör vara $0,2$ mg/L, eftersom det blir fördröjning i mätningen blir verkligt värde efter att luftning stoppats högre än denna skillnad. Ställ in lämpliga värden i närheten av aktuellt uppmätt värde (e.g.: om aktuellt värde är $3,3$ mg/L ställ in låg gräns till $4,0$ och hög gräns till $4,2$; är aktuellt värde $8,5$ ställ in högt värde till $7,5$ och lågt värde till $7,3$; är aktuellt värde $6,4$ ställ in hög gräns till $6,3$ och låg gräns till $6,1$). Detta görs för att undvika att det tar för lång tid att nå ned till valda gränsvärden då den cykliska på/avslagningen av luftning sker.



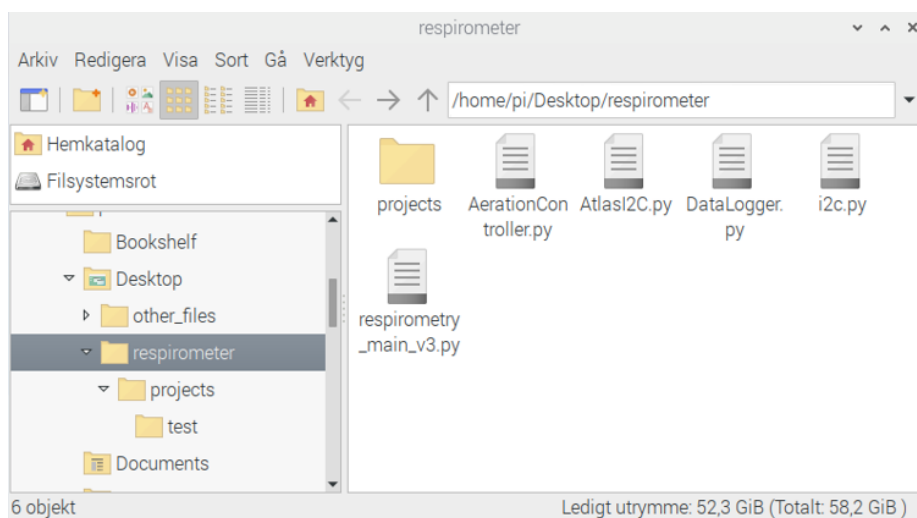
18. Starta testet genom att trycka på ”Start test”.

19. När testet är genomfört avslutas det genom att trycka på ”End test”.

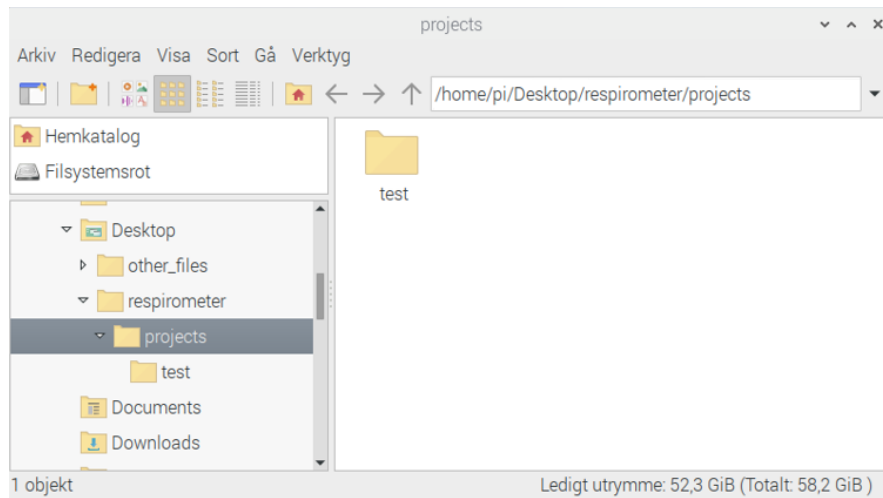
3.5 Exportera data

Data sparas automatiskt i en textfil under försökets gång. För att exportera data till en vanlig PC, följ instruktionerna nedan.

1. Klicka på mappen ”respirometer” som ligger på skrivbordet.



2. Klicka på undermappen ”projects”. Där har en mapp skapats med det testnamn som valdes i samband med respirometriförsöket. I exemplet nedan har testnamnet ”test” valts.



3. För att exportera data, anslut ett USB-minne till en av respirometerns USB-portar. Anslut en mus till den andra USB-porten.
4. Högerklicka på testmappen och välj ”kopiera”.
5. Gå över till mappen för USB-minnet och högerklicka i den tomma ytan och tryck på ”Klistra in”. När mappen har kopierats över, välj ”Eject USB” i övre högra delen av skärmen för att säkert koppla ut USB-minnet. Du kan sedan ta ut USB-minnet från USB-porten.



6. USB-minnet kan sedan kopplas in i en vanlig PC och resultaten föras över dit.