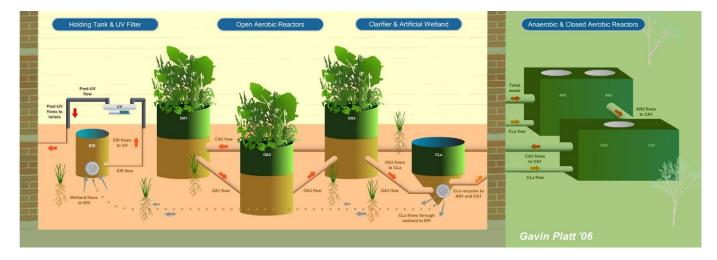
The Living Machine Operator's Handbook '06-'07

Compiled by Audra Abt, Heather Elmer and John Petersen Last Revised JP: 9/3/06



<u>Purpose</u>: This handbook provides procedures and background information for regular operations maintenance, monitoring, and troubleshooting of the AJLC Living Machine. Except for microscopic identification of microorganisms, laboratory procedures for the Living Machine are described in a separate document, Methods for Analyzing Aquatic Ecosystems. If questions arise that cannot be answered here, consult the Living Machine, Inc. Operator's Manual or the appropriate student or faculty listed below.

Emergency and Non-Emergency Contact Information:

Emergency	911
OC Safety and Security	5-8911
OC Facilities Operations	5-8445
Oberlin Police Department	7-774-1061
Oberlin Fire Department	7-774-3211
John Petersen (Faculty Adviser Rm 207)	5-6692. Home: 776-0005, Cell: (440) 935-3415
Cheryl Wolfe (Faculty Adviser Rm 208)	5-5307
Kristin Braziunas (Senior operator)	<u>(206) 920-2325</u>
Zena Grecni (Senior operator)	xxxx

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Safety Procedures and Precautions

This section must be read, understood, and complied with by all Living Machine staff!

The Living Machine is a waste water treatment facility. Hazards associated with wastewater treatment work include physical injury, drowning, infections and infectious diseases, toxic or suffocating gases, electrical shock, and fire¹. All operators should be aware of hazardous situations and equipped to respond in an emergency. Under normal operations, student operators should work with partners to complete job responsibilities.

Specific safety procedure's for AJLC Living Machine staff are outlined below. Additional wastewater and safety issues are described in the pages of the Living Machine Manual provided by Living Technologies. If you are unsure about how to handle a situation in the Living Machine STOP and ask faculty advisors Cheryl Wolfe-Cragin or John Petersen. Notify faculty advisors of ANY Living Machine accident or injury including minor cuts or abrasions. Emergency phone numbers are provided on the cover page.

Physical Injury:

It is particularly important to avoid contact between cuts and abrasions and waste water. Students with open cuts or sores should ensure that these or completely protected from contact, or should avoid working in the Living Machine until wounds have fully closed. A first aid kit is available in the AJLC kitchen to address minor scrapes and cuts.

Drowning:

The closed anaerobic, closed aerobic and final holding tank pose the greatest risk associated with drowning. Operators should be certain to maintain firm footing when working in these tanks, particularly in winter months. The covers to these tanks should be firmly closed and locked down with wing nuts to prevent non-staff members from accessing these tanks. The aerated tanks (both open and closed) present a particular drowning threat to a person working above or near the tanks. Aerated water is less dense than non-aerated water; therefore if a person should fall into a tank they may sink to the bottom.

Electric Shock

Emergency shutoff (red button to left of LM greenhouse doors). Press in case of electric shock within the greenhouse or if electrical cord falls into any of the tanks. Electrical shock frequently causes serious injury. Ordinary 120-volt electricity may be fatal, particularly when experienced in the presence of water. Any electrical system, regardless of voltage should be considered dangerous unless you know positively that it is de-energized. Only qualified and authorized personnel should work on electrical equipment. Personnel must comply with OSHA and local electrical codes and safety regulations.

¹ Some of the materials in this section are excerpted or adapted from Living Machines Incorporated Operations and Maintenance Manual for Living Machine at Adam Joseph Lewis Center for Environmental Studies

Safety Precautions Related to Infectious Disease

Pathogenic and infectious organisms can be found in wastewater. The best protection against contracting infectious diseases such as typhoid, dysentary, hepatitis, and tetanus is personal hygiene. Observe all personal hygiene and cleaning responsibilities. Together we can ensure the Living Machine is as safe and pleasant working environment as possible.

- 1. Operators must be immunized against tetanus
- 2. The single most important measure operators and lab personnel can take to reduce risk of infection or other injury from contact with wastewater is to WASH HANDS with soap and warm water immediately following work before leaving the Living Machine or lab. Thoroughly wash hands after working around the Living Machine and especially before eating or smoking as well as before using the restroom.
- 3. The mini lab sink should be used for hand, goggles, and face shield washing only. Living Machine equipment should be cleaned using the hose or dI water in the greenhouse.
- 4. ALWAYS were protective gloves eye wear, and face shields when there is risk of contact with process fluids, sludges, or any related materials or equipment. Goggles and face shields should be washed with lens cleaner following each use.
- 5. Wash gloves immediately after contact with wastewater. If gloves become soiled please dispose of immediately and use fresh gloves (located in cabinet).
- 6. Shoulder length gloves are located on top shelf of cabinet in case it is necessary to submerge your arm in process fluids.
- 7. Do not wear open toe shoes or sandals in the Living Machine.
- 8. Do not touch pens, logsheets, door handles etc. with gloves or soiled hands.
- 9. Upon finishing operations immediately and thoroughly wash your hands
- 10. If you see a co-worker doing something dangerous point it out to him or her.
- 11. If you are not comfortable performing any task on your own or find yourself confronted with a situation in which you feel unsafe STOP and seek assistance or advice from faculty advisors Cheryl Wolfe-Cragin or John Petersen. Do NOT attempt to address the situation on your own. If none of these individuals are available and you feel the matter is an emergency call security or facilities operations.
- 12. Three kinds of gloves are available in the operations cabinet. Elbow length gloves are the only re-usable variety available in the Living Machine. Standard latex (wrist length) and shoulder length gloves should be disposed of after one use. Re-use of elbow length gloves is contingent upon the cooperation of all operators. NEVER put dirty hands into a pair of gloves. If you believe you have contaminated the inside of a pair of gloves throw them away immediately. If you prefer using a new pair of gloves each time you work you are encouraged to do so.
- 13. NEVER submerse your hands in wastewater deeper than glove length (this includes the effluent sump).
- 14. Maintaining a clean and organized working environment is also crucial to safety in the LM and lab. Be sure to put all materials away when you finish working with them.
- 15. Each day of operations is assigned a cleaning task. Please take care in completing these tasks and consult Cheryl Wolfe-Cragin if you need guidance regarding any of them.

Emergencies:

Many emergencies at a wastewater treatment facility are non-life threatening but have the potential to adversely affect treatment. It is best to take immediate action when these situations arise to avoid complete failure of the treatment system. Some of the more common non-life threatening emergencies are described below.

1) Plumbing Failure:

Breaks or leaks in any plumbing line should be immediately reported to both facilities personnel and to John Petersen and Cheryl Wolfe.

2) Power Failure:

A power failure is serious but not life threatening. The main concern is damage to the process ecology associated with the loss of dissolved oxygen and mixing. When an outage occurs, restore power to the Living Machine as quickly as possible. If power is out more than eight hours, a generator should be brought on line to power the critical components of the system. It is critical that only a qualified electrician connect and disconnect a generator to/from the system.

3) Water Pump Failure:

Pump failures should be addressed immediately but do not constitute a life-threatening emergency. Influent and effluent pumps have duplex units that can be activated manually if necessary. In the event failure of pumps P2 or P3, repair immediately or replace the equipment with like hardware. The makes, models, and suppliers of each pump are listed in the Equipment list at the end of Appendix K and in the Equipment Manual. This list should be kept up to date.

4) Process Failure:

Process failures can include clarifier upsets, loss of dissolved oxygen levels, foaming, odors, overflows, etc. Any of these emergencies may result in discharge of inadequately treated wastewater. Many of these emergency situations and remedial actions are described in Chapter 5 of the Living Technologies manual or in the Trouble Shooting Guide presented in Appendix C of this manual.

Daily Operating Procedures

The Log Sheet (hard copy)

The Living Machine is a complex biological system that has demonstrated its capacity to self-organize and adapt to changing conditions since it was installed in January 2000. The job of the operator is to monitor the system and make sure that it and its supporting equipment are functioning properly. A daily log is kept of key system parameters such as flow rates, dissolved oxygen, temperature, and sludge levels along with checking the mechanized parts of the system so that potential troubles can be quickly spotted and rectified before they become larger problems. The log is also a resource for tracking the long-term behavior of the system. If log sheets run low, photocopy more on the copy machine in Bev's office.

Recording Status of Water and Air Flows

1. Water Flow Meters

Total city water use flow meter

The flow meter that measures total flow of city water into the AJLC is located in the custodian's closet, next to the men's bathroom in the first floor hallway. Minus the little bit of water used in the landscape, all water entering the building is treated by the Living Machine. The key to this closet is in the lockbox stored in the mini-lab with the AV and greenhouse keys. See the **LM coordinator** for the combination to this box.

In the closet, read the large flow meter on the north wall (facing you when you open the door) at about waist height. Record the meter reading in the line labeled Total building flow as well as the previous day's total. Replace the zero (x1) that is written on the device itself with the number indicated by the red needle. This reading yields city water inputs to the AJLC to the nearest cubic foot (1 cf = 7.48 gallons). Calculate daily city water use in the AJLC by subtracting the previous day's number from the current reading. This difference reflects the approximate amount of city water that entered the Living Machine during that period. If Living Machine effluent is being re-used for toilet flush water, the total flow of wastewater into the Living Machine is equal to daily city water use + toilet water use ($h_2OFlush2$)



Magnetic flow meter

The magnetic flow meter is located in the AV room, under the white stairs and above the LM data logger. It measures flow from CA2 to OA1 and effectively equals new water entering the system + recycled water from the Clarifier and OA3. Since we have yet to find a reliable calculation for recycle rates, we must record this number and back-calculate at a later date. Record the LED totalizer (top) reading² on the log sheet and compare to the previous day to determine the volume of water that has entered the greenhouse in this time period.

 $^{^{2}}$ Do not record the magnetic flow meter total when a gpm reading is registering in the bottom of the display, this indicates that water is currently being pumped from CA2 to OA1 through the flow meter. These pumps are generally on for no more than 1-2 minutes. If you notice that the pumps are remaining on for long periods of time, notify Cheryl Wolfe-Cragin.



Air-gap water flow end use configuration

The air gap flow meter is located in the AV room at the foot of the white stairs. It measures city water inputs to the Living Machine and AJLC toilets. Depending upon the orientation of the yellow handled ball valves (Fig. 1,2) this meter measures one of three water uses: LM greenhouse hose water use, make-up water to LM effluent holding tank, or toilet water use. Determine which water use the meter is measuring (see below) and then record the meter reading to the nearest 0.1 gallon. Do not change the position of these valves without consulting John Petersen.

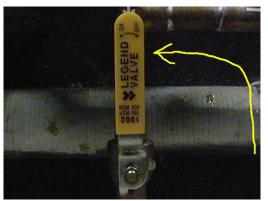


Fig. 1 An open valve is always pointing parallel to the pipe (turned 90° counterclockwise)



Fig. 2 A closed valve is always pointing perpendicular to the pipe (turned 90° clockwise)



1. No water goes through air gap meter

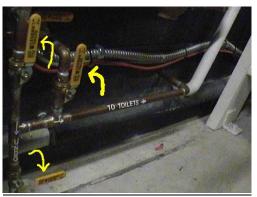


2. Air Gap meter is off and water flows directly to greenhouse hose.

Valve positions and flow meter end-use:



3. Air gap meter is on and green house hose bypass flow is off.



4. Water goes through the air gap meter and then to the greenhouse hose.



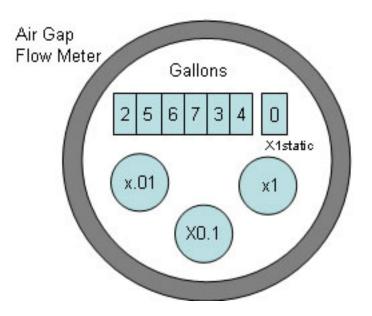
5. Water goes through the air gap meter and off to the toilets (the city bypass valve in the greenhouse is on).



6. Water goes through the air gap meter and off to the underground storage tank.

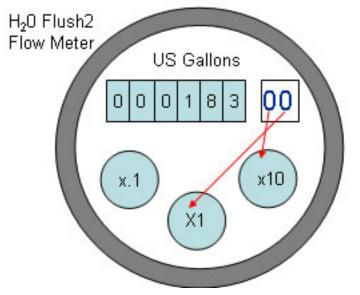
Air gap flow meter

Read and record the air gap flow totalizer to the nearest 0.1 gallon. The last turning digit in the totalizer is tens of gallons. If this digit is between two numbers, round to the lower of the two. The last two digits can be determined by reading the x1 and x0.1 dials.



Effluent flow meter (H₂0Flush2)

 $H_20Flush2$ is located in the greenhouse to the right of the UV filter. It measures Living Machine effluent re-use in the Lewis Center (toilet flush water and landscape irrigation). Record this meter reading to the nearest 0.1 gallon. The last turning digit in the totalizer



2. Blowers, Pressure Gauge and Air Flow Meters

Blowers and pressure gauge:

Six aquarium pumps, located at the easternmost end of the loft above the AV room, are available as blowers to supply oxygen to the aerobic tanks. Two blowers (1,2) are connected to the closed aerobic tanks, while the other four (3,4,5,6) feed into the open aerobic tanks. On the wall above the pumps are switches for each with lights that indicate which blowers are on.



Confirm which blowers are operating (the red light indicates there is power to a particular blower and vibration of the equipment indicates its on) and circle on log sheet. Check recent logsheets to see if the configuration has changed. If a light has burnt out, or a blower is off that should not be or is very hot, notify **Cheryl Wolfe-Cragin**. Also check the messages board in the greenhouse, as someone else may have altered the blower arrangement for an experiment or in response to TSM results (see TSM section.)

To the right of the blowers is an air pressure valve with a circular dial. This dial indicates the total air pressure for the open aerobic system. Record on the log sheet next to the blowers what the total air pressure is from the blowers, rounding to the nearest tenth. Typical air pressure ranges from 2.5-3.5psi. If the gauge reading is significantly different contact **Cheryl Wolfe-Cragin**.

Air flow meters:

The meters for the open aerobic tanks are located on the wall behind OA3. From left to right, the meters correspond to OA3, OA2, and OA1, respectively. Tap the plastic tube a couple times, as the 'hammer' will sometimes get lodged in the tube. Approaching the gauge at eye level, record where the largest diameter of the float is sitting as the air pressure for the blowers. Air flow meters will temporarily read zero when the clarifier recycle pumps are operating, wait until they are finished (1-2 minutes) and then read air flow. Air flow meters will also read zero when TSM measurements are in process 10-11am and 4-5pm. Operations should not be completed during these times.



[Note: Need better image of float!]

The closed aerobic meters are in the AV room in the dark cove across from the bottom of the stairs. A small flashlight stored by the message board will help in reading the meters. The CA2 meter is on the left with CA1 just to the right of it. Read these meters in the same manner as above.

Don't adjust airflow unless you are sure that aquarium pumps are working, and then only do so in consultation with **John Petersen or Cheryl Wolfe-Cragin**. To alter airflow, open or close the orange valve below the meter. The valves are fully open when the orange handle is parallel to the inlet and outlet and completely closed when the handle is at a 90° angle. Keep in mind that adjusting one valve will cause the other(s) to change as well in the opposite direction. If a decrease or increase in airflow is desired across the board, the number of blowers in use (see Blowers above) must be adjusted. Do not, under any circumstances close the orange pressure release valve located below the air flow gauges in the LM greenhouse as this will cause the blowers to over-heat.

Normal operating levels: CA1: 0.3-1.5cfm CA2: 0.3-1.5cfm OA tanks: .0.2-1cfm

Assessing in situ Water Quality with Hand-Held Equipment

1. Dissolved Oxygen and Temperature (YSI model 55 probe)

Dissolved Oxygen (D.O.) is measured manually with the YSI 55 D.O. Probe. We also have an automated D.O. monitoring system that is described in Section 11 of this manual



Storage and Preparation:

Turn on meter and wait 15 minutes. The probe should be stored within the sleeve inside the body of the meter. There should be a damp sponge within this sleeve to maintain an environment of 100% humidity. If the sponge is dry, moisten it. Dissolved oxygen calibration must be done in an environment with a known oxygen content. Since the amount of oxygen in the atmosphere is known, it makes an excellent environment for calibration (at 100% relative humidity). The calibration/storage chamber contains a moist sponge to create a 100% water saturated air environment. Calibration of the YSI 55 DO meter should be performed with the probe in this chamber. Remember that the probe chamber must be kept moist (not wet), and that the probe must be inserted for calibration. Refer to YSI 55 maintenance section for more information.

Calibration:

- 1. Simultaneously push the up and down arrow keys. Hold and release.
- 2. Set the altitude (800 ft). The display will read 8, press Enter.
- 3. Wait for the meter reading to stabilize and press Enter again.
- 4. Set salinity. The display should read 0 (ppt = parts per thousand), if it doesn't, use the arrow keys to adjust it. Press Enter when complete.
- 5. The probe is now calibrated and ready for use.
- 6. D.O. should be read in mg/L. Press Mode until this type of reading is displayed.



Operation:

- Start at the Effluent sump and work backwards to the Marsh sump and then OA1, taking
 readings on the west side of each tank. Raise and lower the probe at a rate of 1ft /sec in order to
 prevent the exhaustion of D.O. at the membrane (the probe consumes a small amount of
 oxygen). White tape marks on the cord near the probe are spaced 1 ft apart to help gauge this.
 While dunking the probe at 1 ft/sec, wait until the reading is stable to within 0.3-0.5 mg/L and
 record the D.O. plus temperature (celsius bottom right corner of meter display) on log sheet.
 In order to get consistent readings it is important that readings are all taken from the same place
 and everyone use a similar technique for moving the probe up and down.
- 2. When finished measuring the 3 tanks, gently hose off the probe and cable, making sure to soak the sponge on which the membrane sits during storage. Remember that the probe chamber must be moist (not wet), and that the probe must be inserted for calibration. Refer to YSI 55 maintenance section for more information.

Replacing the membrane and electrolyte:

The membrane and electrolyte should be replaced at the start of each semester and whenever the probe can not be calibrated or when response time to changes in dissolved oxygen is slow or erratic. Different YSI probes require different kinds of membranes. Three different types of membranes are available for our YSI model 55 probe. In the LM we use the Model 5775 Standard Membrane Kit. This kit contains thirty 1 mil (.001") membranes and a bottle of KCl solution.

Replacement membranes are located in the DO probe maintenance toolbox (yellow). To replace the membrane:

- 1. Unscrew the probe sensor guard.
- 2. Remove the old O-ring and membrane.
- 3. Thoroughly rinse the sensor tip and KCl reservoir with distilled water.
- 4. Prepare the electrolyte according to the directions on the KCl solution bottle.

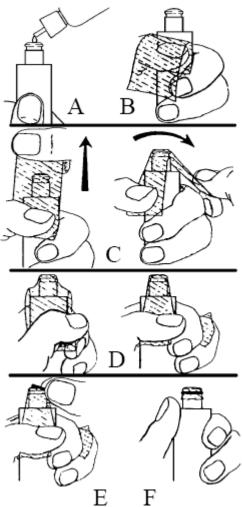
- 5. Secure a membrane between your thumb and the probe body. Add electrolyte to the probe until a large meniscus completely covers the gold
- cathode. Note: Handle the membrane material with care, touching it at the ends only.
- 6. With the thumb and forefinger of your other hand, grasp the free end of the membrane.
- 7. With a continuous motion, stretch the membrane up, over, and down the other side of the sensor. Stretching forms the membrane to the contour of the sensor tip.
- 8. Secure the end of the membrane under your forefinger while continuing to hold the probe.
- 9. Roll the O-ring over the end of the probe, being careful not to touch the membrane surface. There should be no wrinkles in the membrane or trapped air bubbles under the membrane. Some wrinkles may be removed by lightly tugging on the edges of the membrane beyond the O-ring.
- Trim off excess membrane with manicure scissors (DO probe maintenance toolbox). Check that the stainless steel temperature sensor is not covered by excess membrane.
- 11. Shake off excess KCl. Rinse the stainless steel thoroughly with distilled water to prevent corrosion.
- 12. Reinstall the sensor guard. The sensor should be kept in a humid environment (such as the calibration chamber) between measurements and when not in use.

2. pH and Conductivity (YSI model 63 probe)

The YSI 63 probe is used to measure pH, conductivity/salinity and temperature. This probe must be calibrated daily before being used to take pH measurements.



<u>Six modes of operation</u>: 1) pH -- Displays pH and temperature (°C).



2) Conductivity -- A measurement of the conductive material in the liquid sample without regard to temperature. Also displays temperature(°C).

3) Specific Conductance -- Also known as temperature compensated conductivity which automatically adjusts the reading to a calculated value which would have been read if the sample had been at 25°C (or some other reference temperature which you choose). Also displays temperature (°C).

4) Salinity-- A calculation done by the instrument electronics, based upon the conductivity and temperature readings. Also displays temperature (°C).

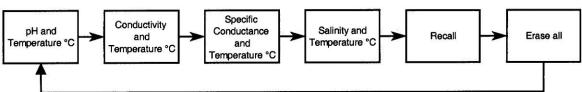
5) Recall -- Allows previously stored data to be displayed.

6) Erase all -- Allows ALL previously stored data to be deleted.

To change between the Model 63 modes, simply press and release the MODE key.

Mode	Display
рН	pH
Conductivity	μS or mS
Specific Conductance	μ S or mS and flashing °C
Salinity	ppt
Recall	rcl
Erase All	EraS

Parameters measured for daily LM ops





Probe Storage:

For short term storage between measurements in the field (up to one week), place the probe in the transport chamber in the side of the instrument case. Make sure that the sponge inside the chamber is wet (tap water).

For long term storage (over one week), place the probe in the storage bottle (provided) containing a mixture of 50% pH 4 buffer and 50% 1.5M KCl. This will assure the fastest possible pH response. Do NOT store the probe dry or in distilled or deionized water. If pH4/KCl storage solution above is not available, store the probe in tap (NOT distilled or deionized) water.

After storage in the pH 4/KCl solution described above, place the probe in the transport chamber in the side of the instrument case or soak the probe in pH 7 buffer for 5 to 10 minutes allowing it to acclimate before calibrating.

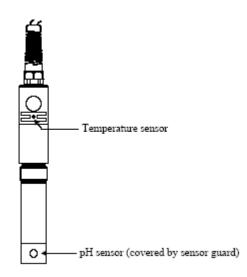
If the probe has been inadvertently left in air and the reference electrode junction has dried out, good function can usually be restored by soaking the probe in the pH4/KCl solution described above.

One-point pH calibration (used for normal daily operations):

This probe must be calibrated before being used to take pH measurements. A three point pH and one point conductivity calibration is performed by the equipment maintenance operator each Monday. A one point pH calibration is performed each day during operations. pH standard solutions contain ingredients that may be hazardous to your health. Avoid inhalation, skin contact, eye contact or ingestion. Use gloves provided and refer to attached material safety data sheet for more information.

The accuracy of pH measurements can be maximized by calibrating as close as possible to sample temperature and by allowing sufficient time for equilibration with the temperature of the buffer.

- 1. Turn the instrument on by pressing ON/OFF and allow it to cycle through its self test sequence.
- 2. Rinse the probe with DI water and dry it carefully.
- 3. Immerse the probe in pH 7 standard making sure that both the pH and temperature sensors are covered by the solution (See figure below).
- 4. Use two fingers to press and release both the up and down arrow keys on the meter at the same time. The display will show CAL at the bottom, STAND will be flashing and the pH reading will show 7.00 (the buffer to be used to adjust the offset).



NOTE: The model 63 automatically accounts for the fact that the true pH of the buffers changes with temperature, therefore, the pH values displayed during calibration will vary with temperature. For

example, a pH 7 buffer at 20°C (rather than 25°C) has an actual pH of 7.02 and this number (rather than 7.00) will appear on the display when the probe is placed in the solution.

- 5. Press ENTER. CAL will appear at bottom of display, STAND will stop flashing and the pH calibration value will appear with a flashing decimal point.
- 6. When the reading is stable the decimal point will stop flashing. Press and hold ENTER to save the calibration point. SAVE will flash on the display along with OFS to indicated that the offset value has been saved.
- 7. SLOPE will now appear flashing on the display. This indicates that the slope is ready to be set using a second pH buffer. The system is now calibrated at a single point (pH 7)
- 8. Press the mode key to return to normal operation.
- 9. Rinse the probe with DI water and then dry it carefully using a kim wipe. Proceed to making measurements.

Three-point pH calibration (conducted once per week):

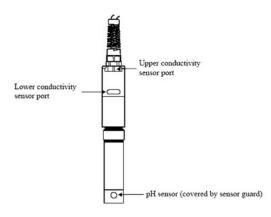
Three point calibrations should be conducted once per week. Follow the procedure described above through step 7 and then proceed with the following:

- 1. Place the probe in bottle of pH4 standard. Make sure the temperature sensor is immersed in the solution.
- 2. Press ENTER. The display should show CAL at the bottom, SLOPE will stop flashing and the pH calibration value is shown with the LEFT decimal point flashing.
- 3. The reading is stable when the decimal point stops flashing. Press and hold ENTER to save the first slope. The display will flash SAVE along with SLP to indicated that the first slope value has been saved.
- 4. SLOPE will start flashing again indicating that the slope is ready to be set using a third pH buffer.
- 5. Rinse the probe with dI water and dry carefully.
- 6. Place the probe in pH 10 standard. Be certain the temperature sensor is immersed in the solution.
- 7. Press ENTER. CAL will appear at the bottom of the display, SLOPE will stop flashing and the pH calibration value is shown with the RIGHT decimal point flashing.
- 8. When the reading is stable (decimal point stops flashing) press and hold ENTER to save the second slope. SAVE and SLP will flash on the display to indicate that the second slope value has been saved.
- 9. Rinse the probe with dI water and dry it carefully.
- 10. The system is now calibrated at three points and will return to normal operation.

Conductivity calibration (conducted once per week):

This calibration should be performed by the equipment maintenance operator weekly.

- 1. Turn the instrument on and allow it to complete its self test procedure.
- 2. Clean the conductivity cell by dipping in water or Dow chemical bathroom cleaner and agitate for 2-3 minutes. Use the nylon conductivity brush to dislodge any contaminants from inside the electrode chamber. Repeat until the cell is completely clean.
- 3. Rinse the cell thoroughly in deionized, or clean tap water.

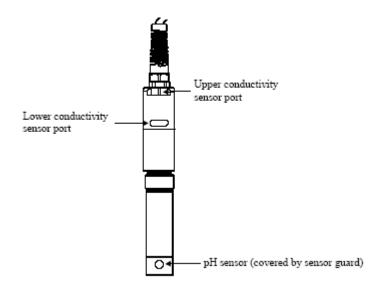


- 4. Press MODE to advance the instrument to conductivity.
- 5. Insert the probe into conductivity calibrator deep enough to completely cover the probe. Both conductivity ports must be submerged (See Fig. 2).
- 6. Allow at least 60 seconds for the temperature reading to stabilize. (Perform conductivity calibration at a temperature as close to 25°C as possible. This will minimize any temperature compensation error. If it is below 70°C in the Living Machine perform this procedure in the lab/AV/atrium after the conductivity calibrator has equilibrated to 25C.
- 7. Move the probe vigorously from side to side to dislodge any air bubbles from the electrodes.
- 8. Simultaneously press and release the UP/ DOWN arrow keys.
- 9. CAL will appear in the display to indicate that the instrument is in calibration mode.
- 10. Use the UP/DOWN arrow keys to adjust the reading on the display until it matches the value of the calibration solution you are using. Once the display reads the exact value of the calibration solution being used (the instrument will make the appropriate compensation for temperature variation from 25 °C) press ENTER. SAVE will flash across display indicating that the calibration has been accepted.

Making measurements:

When making field measurements, store the probe in the transport chamber with tap water moistened sponge. Do not leave probe out in air as this will dry out the reference electrode junction.

- 1. Insert probe into west side of each tank.
- 2. Completely submerse probe into the tank to ensure that pH, temperature, and conductivity sensors are all covered by liquid (see figure below).
- 3. Shake gently to remove any trapped air bubbles and then hold probe very still until the pH reading stabilizes³.
- 4. Press mode once to display conductivity, verify the reading is stable and record.



Storing and recalling data from meter memory:

To avoid confusion, it is wise to immediately record data collected on paper. However, it is possible to temporarily store data in the electronic memory of the meter and then transfer this information to the operation sheet later.

³ The first pH reading after storage in buffers may take 5-10 minutes to stabilize.

- 1) While a pH, conductivity, specific conductance or salinity reading is displayed on the screen press and hold ENTER for 2 seconds. SAVE will flash on the display along with the current site identity (1-50) being used.
- 2) When all 50 sites contain data, FULL will flash on the display. This message will remain on the screen (even if the instrument is turned off) until a key is pushed.
- 3) Press any key to acknowledge that the memory is full. Upon this any subsequent saved data will begin overwriting existing data with site #1.Record all data on LM log sheet once finished taking measurements.
- 4) To recall data, Press MODE until rcl and Site ID are displayed.
- 5) Press ENTER to review the last set of saved data. The display will show pH and temperature. Press ENTER to move through each measurement mode for a given site ID.
- 6) Press UP/DOWN arrow keys to move up and down through saved sets of data.
- 7) When desired Site ID is displayed, press ENTER to display data. Press ENTER to move through each measurement mode for that site.
- 8) After recording all measurements on LM log sheet, press MODE until ErAS is displayed on the screen.
- 9) Press and hold DOWN arrow and ENTER simultaneously for 5 seconds until the display reads dOnE. This erases all saved data, be sure all information is recorded on log sheet before you erase.

pH sensor cleaning

Cleaning is required whenever deposits or contaminants appear on the glass pH sensor. Error messages such as 'undr' or 'ovr' during calibration or measurement also indicate that the sensor may need to be cleaned. Inform the equipment maintenance operator and faculty supervisors if you encounter these messages or slow response/failure to stabilize, they will take care of cleaning the sensor. The cleaning procedure described below should be performed by equipment maintenance operator or faculty supervisors only! If the following instructions are not sufficient, please refer to complete YSI 63 operations manual (pdf) on operations computer.

- 1. Unscrew and remove the small guard that protects the pH sensor. Use tap water and a clean cloth or lens cleaning tissue to remove all foreign material from the glass sensor. Great care is required to prevent breaking the glass sensor bulb. **DO NOT** place sensor upright in any container without guard around bulb, it **WILL** break!
- 2. Reinstall the small guard that protects the pH sensor.
- 3. If good pH response is not restored by the above procedure, remove the guard and perform the following additional procedure:
- 1. Soak the probe for 10 to 15 minutes in clean water containing a few drops of commercial dishwashing liquid.
- 2. GENTLY clean the glass bulb by rubbing with a cotton swab soaked in the cleaning solution.
- 3. Rinse the probe in clean water, wipe with a cotton swab saturated with clean water, and then re-rinse with clean water.
- 4. Reinstall the small guard that protects the pH sensor.
- 5. If good pH response is still not restored by the above procedure, remove the guard and perform the following additional procedure:
- 6. Soak the pH sensor for 5 minutes in one molar (1 M) hydrochloric acid (HCl).
- 7. GENTLY clean the glass bulb by rubbing with a cotton swab soaked in the acid.
- 8. Rinse the probe in clean water, wipe with a cotton swab saturated with clean water, and then re-rinse with clean water.
- 9. Reinstall the small guard that protects the pH sensor.

- 10. If biological contamination of the reference junction is suspected or if good response is not restored by the above procedures, remove the guard and perform the following additional cleaning steps
- 11. Soak the probe for approximately 1 hour in a 1 to 1 dilution of commercially-available chlorine bleach.
- 12. Rinse the probe with clean water and then soak for 1 hour in clean water to remove residual bleach from the junction.
- 13. Reinstall the small guard that protects the pH sensor.

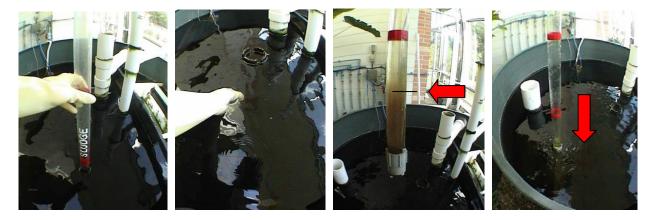
Conductivity sensor cleaning

The single most important requirement for accurate and reproducible results in conductivity measurement is a clean cell. A dirty cell will change the conductivity of a solution by contaminating it. The following procedure should be used for cleaning the conductivity sensor:

- 1. Dip the cell in cleaning solution and agitate for two to three minutes. YSI recommends foaming acid tile cleaners such as Dow Chemical Bathroom Cleaner. When a stronger cleaning preparation is required, use a solution of 1:1 isopropyl alcohol and one molar (1 M) hydrochloric acid (HCl). Remove the cell from the cleaning solution.
- 2. Use the nylon brush (supplied) to dislodge any contaminants from inside the electrode chamber.
- 3. Repeat steps one and two until the cell is completely clean. Rinse the cell thoroughly in deionized, or clean tap water.

3. Clarifier Sludge Depth

Wastewater from the last open aerobic reactor (OA3) flows into the clarifier. The purpose of this tank is to settle and concentrate the biosolids that have been generated in the upstream treatment units. To measure depth of sludge in the clarifier, use the "sludge judge," a 6-foot-long clear plastic tube that's stored against the wall near the door leading outside. Red tape marks every foot of tube as a reference for you. Stick the sludge judge into the clarifier on the south side of the tank, about half a foot from the center pipe. Feed the tube down until it touches the bottom (it should be almost totally submerged when this finally happens), and then draw it back up. Estimate how many inches of sludge are at the bottom and record this. Empty the sludge judge by depressing the white tab on the bottom against the inside rails of the clarifier.



Clarifier sludge troubleshooting⁴:

⁴ Refer to Operations and Maintenance Manual 2-3:2-5 and 5-11:5-15 for complete discussion of clarifier settling goals and troubleshooting.

The type of bacteria we are trying to cultivate for carbon and nutrient digestion are referred to as 'floc formers' because they have the tendency to conglomerate and eventually settle out of the water column. Filamentous bacteria are also commonly present but do not settle well in a clarifier. If filamentous bacteria become the dominant organism in the Living Machine this can prevent the floc formers from congregating and settling.Floc-forming suspended biosolids are critical to system performance.

Solids recycle from the clarifier enhances the settleability of suspended biosolids by favoring the growth of floc-forming bacteria. If there is an unsettled fraction of biosolids or high turbidity visible in the water column above the settled sludge in the clarifier, then recycling of biosolids from the clarifier should be increased, as long as there is a measurable sludge blanket in the clarifier bottom, to increase the dominance of floc-forming bacteria. If there is no measurable sludge or solids in the clarifier bottom, the recycle rate should be decreased to allow time for floc-forming bacteria to settle. The ideal recycle rate is achieved when the solids inventory in the clarifier is low and the settleable solids volume (SSV) in the last open aerobic tank is between 10% and 30%.

Solids bulking and rising:

Solids on the surface of the clarifier can cause a major system upset and should be dealt with immediately. The types of solids on a surface of the clarifier can be divided into two categories: Rising solids and bulking solids. If the sludge at the bottom of the clarifier is too old and has gone anaerobic, the production of nitrogen gas from inorganic nitrate (NO3) (which can occur only in anaerobic conditions) can release gas bubbles that break up sludge at the bottom. Chunks of sludge will float to the surface then and the water that enters the wetland will not be clean. A distinctive and often unpleasant odor will often accompany an event such as this, so use your nose as well as your eyes to identify what's wrong.

Another cause of floating sludge could be an unhealthy bacterial composition in the system. If predominance of filamentous bacteria is the reason for surface biosolids, oxygen should be lowered in the whole system because filamentous bacteria cannot survive in low oxygen conditions. Floc formers (facultative aerobes) will go dormant or continue to live through this as long as it's not maintained for too long.

4. Turbidity

Measure turbidity using the Model 711 Suspended Solids meter found in the Everyday Needs box on the table. Place the sensor in the effluent sump and press ON. Make sure the sensor is in g/L mode. The reading should appear and stabilize within 30 seconds. Record this on the log sheet.



Automated Equipment: Maintenance and Troubleshooting

1. Automated Dissolved Oxygen and Total System Metabolism (TSM)



Probes are used to continuously monitor dissolved oxygen (DO) and temperature in tanks CA1, CA2, OA1, OA3, the clarifier and the pond [pond not implemented yet]. These provide real-time information about ecological/metabolic conditions throughout the Living Machine. Total system metabolism (TSM) is measured by tracing changes in dissolved oxygen when bubblers are turned off. Because aerobic microbes in the tanks consume oxygen as they process organic matter and ammonium, the amount (concentration) of dissolved oxygen goes down when bubbling is turned off. The rate at which it declines is a measure of how much reactive organic matter and ammonium are present. Bubblers for the CA and OA tanks are programmed to shut off automatically for 45 minutes at 4am, 10am, 4pm, and 10pm. Operations should not be completed between 10-11am and 4-5pm because probes cannot be removed for daily maintenance during these times.

Daily maintenance of TSM probes and mixers:

The probe's membrane must be kept free from deposits. All surfaces in a biologically active system such as the Living Machine will be covered by a film which acts as a barrier to the oxygen that must diffuse through the membrane. The membrane *MUST* be cleaned daily. Cleaning can be performed with a damp cloth or soft paper. The membrane is strong, but care must be taken when cleaning. Wear gloves and a face shield when cleaning probes to protect your eyes, nose, and mouth from splashing wastewater!

1. Remove each probe from its tank by pulling the string or plastic handle attached to the probe holder (not the blue/black data or power cable) and separate it from the white mixer assembly. Spray CA1 and 2 probes with hose while they are on their way up but still below the top of the manhole. Do NOT pull directly on the cable, even if it's difficult to remove the probe. Instead, grasp the fitting at the base of the cable so as not to damage the electrical connections. Minimize splashing by pulling the probe downward to remove from the mixer. Notify the operations coordinator if you can't separate the probe from its holder.

2. Clean one probe at a time to avoid switching tanks when you replace them. To clean CA1 and CA2 probes, fill the sludge bucket with water. Submerse probe in the bucket and clean probe body

and mixer housing with scrub brush. Gently wipe membrane with gloved finger or damp paper towel. DO NOT use brush on probe membrane. Remove stir bar and wash off the spin plate. Inspect stir bar for signs of wear (black magnet exposure) and replace if necessary from stock in yellow DO probe maintenance toolbox. If you accidentally lose a stir bar or discover one missing from any motor assembly please replace. Note all stir bar changes in TSM section of LM log. Notify the LM coordinator if the stir bar supply runs low. Dump bucket back into to the closed aerobic reactor, rinse with the hose a few times and dump into CA again. Follow same procedure to clean OA1, OA3 and clarifier probes but use a bucket from the greenhouse or simply spray them with greenhouse hose.

3. Thoroughly inspect the mixing motor. Inspect the junction between cable and mixer motor. Look closely for hairline cracks in the cable. Examine glass stir plate on mixing motors for the presence of condensation inside the motor body. If you notice any of these conditions, remove the mixing motor from the tank immediately. The mixer motor WILL break if the housing leaks. The best way to avoid this is to remove suspect motors from tanks so they can be repaired rather than replaced. Record this information in the LM log and notify John Petersen immediately. If you remove a mixing motor for repairs you must be certain that the DO probe (the round black probe) is placed into the calibration bucket or back into the tank. If the DO probe is exposed to the air for a prolonged period it will need to be rebuilt. Record whatever action you take in the log.

4. Thoroughly inspect the DO probe. Look closely for hairline cracks in the cable near where it enters the probe – this is where failure generally occurs. Examine the DO membrane (the white part), looking for cracks and creases. Damaged membranes need to be replaced. Report any problems with the DO probe to the LM coordinator. Probes should not be left out of water unless you are certain that the cable is cracked.

5. If no problems are observed, reattach the probe to its motor and replace in the appropriate tank in approximately the same location. Each probe needs to go back into the tank it came from or our data will be hopelessly confused. Replace worn spin bars with new ones located in the DO probe kit toolbox.



Cables from DO probes and mixer assemblies are plugged into the orange "TSM junction box" on the center of the North greenhouse wall. Inside this box, the small 'BNC' type connectors are for the signal from the DO probe, the large microphone-type connectors are for temperature and mechanical mixers. The TSM junction box is in turn connected to a data logging computer on the other side of the wall which receives and transmits sensor data to the AJLC server. DO probes require continuous mixing to function properly. Light bulbs in this junction box indicate the status of TSM motor circuits. Bulb filaments for plugs 1-5 should be partially lit. An extra bright filament indicates the mixer motor is shorting out and needs to checked for failure. (See #3 above). If the motor is malfunctioning, the cable worn or cut, or condensation behind the glass stir bar plate, remove the mixer motor from its tank and immediately notify John Petersen.

Plug Lo	cation	Tank
1	CA1	
2	CA2	
3	OA1	
4	OA2	
5	OA3	

Calibrating the dissolved oxygen probes (once/week):

The TSM dissolved oxygen probes need to be calibrated once per week. This is the responsibility of one operator chosen at the beginning of each semester. Calibration can not be done during times when TSM readings are being made and the tanks are not aerated (i.e. not between 10:00-11:00am, 4:00-5:00 pm, 10:00-11:00pm and 4:00-6:00am). Calibration should be performed at least 30 minutes after the conclusion of the previous TSM measurement. This allows the calibration bucket to become resaturated with oxygen after the TSM measurement ends. The following procedure should be used for calibration:

- 1. "LoggerNet"software on the AJLC server in the interns office is used to calibrate the probes. This software needs to be running continuously (24 hours a day) to collect data on all of the different sensors installed throughout the AJLC. This program must be left on when you are finished with calibration or data collection will stop. For calibration, you need to open the LogerNet "connect" window.
- 2. Select Imdatalogger from the station list.
- 3. Open ports/flags dialog box. (No boxes should be checked in either list)
- 4. Click on 'start cal' (flag#1) from the flags column. The box should turn black, be certain that the box is black and not grey. This tells the system to flag data from the do probes with a different ID, indicating that the data being recorded from these probes is no longer from the LM tanks.
- 5. Remove all probes and their mixing motors with associated associated temperature monitoring probes from their tanks and clean them thoroughly. Once they are clean, place them all in the bucket of oxygen-saturated water next to OA3. This bucket should have an airstone in it and should have been vigorously bubbled for at least the previous 24 hours. It is absolutely crucial that the OA3 thermister be submerged in water within this bucket and functioning properly; temperature data from this sensor is used for calibration. It is also critical that each TSM probe/mixer assembly is placed in bucket or tank such that the probe membrane is facing up (same orientation that they are in tanks).
- 6. In the LoggerNet connect window, press 'data display Graph 3' button. This is a graph of actual dissolved oxygen being measured every 10 seconds.
- 7. Verify that appropriate inputs are selected in setup at bottom of graph 1 window. DO1 through DO5 mg/L should be selected.
- 8. Wait until the dissolved oxygen readings on the computer have stabilized (at least 10-15 minutes). Stabilized means that you see readings as a relatively straight line in the graphs. Once stabilized the probes should be reading similar concentrations of dissolved oxygen ranging between 8-10mg/L. Gross deviations in dissolved oxygen measurement or failure of DO readings to stabilize may be indicative of a probe malfunction. Please notify John Petersen if you notice such a pattern in the dissolved oxygen graph. Wait until all DO readings are stable, wherever that stability may be. Accuracy of calibration depends primarily on the probe having reached equilibrium with the temperature at which you are calibrating. Be sure to give the probes adequate time to stabilize before continuing.
- 9. Once DO readings from all probes are stabilized select 'lock cal' (flag#2) in the flags column of ports/flags window. This calibrates the probes by assigning a unique calibration constant to

each one. After 15 seconds all probes should read equal dissolved oxygen concentrations (graphs should converge).

- 10. Once this has occurred, deselect "lock cal" (flag #2).
- 11. Move probes to low dissolved oxygen bucket (no aeration stone) filled with water from custodial closet or outside hose without sprayer. You want to fill this bucket with as little splashing of water as possible to as to avoid aerating it. I the probes are operating properly, you should see the graph of dissolved oxygen concentration go down to similar levels for all of the probes. Note any probes that respond differently
- 12. Replace all probes in their appropriate tanks. Do not switch cable locations in orange DO junction box and be sure to return probes to the correct tanks. It is imperative that probes be returned to the correct tanks or the data collected will be entirely confused.

Input # in orange box:	Tank
1	CA1
2	CA2
3	OA1
4	OA3
5	Clarifier

- 13. Once probes are all back in their tanks, unclick 'start cal' (flag#1) and close the ports/flags window. You can minimize the PC208 program window but do not quit the program window. PC208 must always be running for data to be downloaded to the server.
- 14. Empty, scrub out, refill and replace the airstone in the bucket of water used for calibration. It takes a while for it to become saturated with oxygen. The bucket should be cleaned and refilled immediately after calibrating so it is ready for the following week. Exception: During summer months, bucket can be cleaned and left empty and re-filled one day prior to the next calibration to avoid algae growth. NEVER close pressure release valve or even turn it down significantly. This causes the blowers to over-heat. If more air flow is required run an additional blower.
- 15. Record what you've done in the Living Machine operations database. Add to the bottom of today's record or start a new one if operations have not been done yet. Document the calibration in the datasys3 event log as well, noting any problems or changes to the system.
- 16. The status of the TSM system can be determined by referring to the Access Sysmode in the Lmhour and minute tables. Sysmode 2: Probes are out of tanks for calibration or maintenance. Sysmode1: Probes in tanks, TSM is in progress (aeration should be off). Sysmode 0: Probes in tanks, Aeration on. Activating Flag#1 prior to removing probes from tanks puts the system in Sysmode2.

Troubleshooting DO probes and TSM:

If bubbling is not being delivered to the tanks during normal operations, the most likely cause is malfunctions with the TSM system. Notify John Petersen.

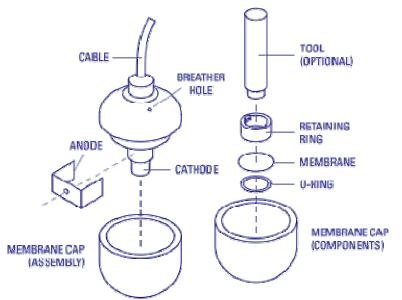
If mixing motors will not spin, first check that the cable is plugged in within the orange box. Contact the LM coordinator if this does not solve the problem. Within the building's main electrical panel, power is supplied to the mixers through circuit breaker #48. Follow the procedure outlined below under, "Checking Web Data"

Renovating dissolved oxygen probes

When the dissolved oxygen probes are damaged, fail to calibrate, drift over time, produce variable output or respond very slowly to changes in oxygen they must be rebuilt. Oxygaurd, the manufacturer

of these probes recommends that membranes not be changed unless a problem of this sort is evident. The following procedure should be followed for probe renovation:

- 1. Wearing gloves and face shield, rinse the probe in water and wipe clean.
- 2. Unscrew the cap (by hand), dump electrolyte (liquid), and rinse the probe internals with tap water. Save the black o-ring.
- 3. Inspect the anode. Clean off any LOOSE, white deposits with a non-metallic nylon nail brush located in DO probe maintenance toolbox. Do not scrape the anode with any metallic object. If the anode is very corroded, replace it. Anodes last approximately 4 years⁵. Check that the nut under the anode is tight before fitting a new anode.
- 4. Inspect the cathode and remove any deposits using wet or dry emery paper, 400 grit. The cathode should not be polished.



- 5. Clear vent hole in top of probe of deposits by inserting and removing a needle. Inspect large O-ring between cap and probe body and replace if damaged. Although the black DO probes all look the same, we actually use probes with two different designs. One has course threading and a fat o-ring that goes on before the body is assembled when the membrane is changed, the other has fine threading and a thin o-ring that goes on After the body is assembled when the membrane is changed. The probe and cap will be destroyed if someone tries to mix the cap from one unit with the base of another. Replacement cost is approximately \$400.
- 6. Unscrew retaining ring in membrane cap (with penny). Remove and discard old membrane and membrane o-ring.
- 7. Thoroughly dry probe cap and retaining ring with paper towel.
- 8. Install new membrane o-ring using tweezers, ensure it is properly seated around membrane opening.
- 9. Install new membrane using tweezers followed by the retaining ring (use penny to screw down).
- 10. Inspect the newly installed membrane from the probe exterior, it should contain no wrinkles or creases.

⁵ Living Machine oxyguard probe anodes were replaced 8/04. Point Four Systems, the manufacturer of oxyguard DO probes, sells two versions of this probe that are designed to measure different ranges of DO concentration. These probes differ in their anode material and use different electrolyte recipes. John Petersen's probes are Type I and contain a zinc anode. Type II probes contain a lead anode and measure a narrower range of DO concentration.

- 11. Fill newly renovated membrane cap to the brim with electrolyte (do not shake electrolyte immediately prior to use as this creates air bubbles) and fit it to the upper part by holding the upper part with the vent hole away from you.
- 12. If renovating probe style with wide threads and fat o-ring between two probe body and cap examine o-ring and replace if necessary.
- 13. Engage the cap with the upper part and slowly screw the cap into place without turning backwards at any time. Ensure that the O-ring seats properly. Excess electrolyte should spray out the vent hole.
- 14. Tighten the cap firmly by hand. Inspect the membrane for air bubbles, shake the probe next to your ear, you should not be able to hear electrolyte sloshing in the probe body.
- 15. If renovating probe style with fine threads and thin o-ring between two halves of body, reinstall o-ring by rolling over probe body until it is seated in groove.

Electrolyte Recipe⁶

Add 125 grams Sodium Chloride (NaCl) to one liter of liquid made up of 2 parts distilled water and 1 part glycerin. (The glycerin makes the electrolyte a bit more viscous which aids probe stability and reduces electrolyte loss through breather hole). ALTERNATIVELY- If glycerin not available. Add 125 grams Sodium Chloride (NaCl) to one liter of distilled, (or deionized water). NaCl must be laboratory grade. Do not use salt from supermarket which has iodine added.

2. In Situ Conductivity Probe

Electrical conductivity (EC) is a measure of a water or solution's ability to conduct an electrical current. Because electrical current is transported by the ions in solution, conductivity is a measure of the amount of ions present in solution. Measured EC value can be used as a surrogate measure of total dissolved solids (TDS). Many ions are removed from the water as it is treated by the Living Machine, so the conductivity of the effluent provides one good measure of the treatment process. EC is measured in millisiemens per meter or centimeter (mS/cm or M).⁷ Operators manually measure conductivity of Living Machine effluent using the YSI 63 meter (see above). Effluent conductivity is also measured *in situ* (in place) in the effluent sump by a conductivity probe that continuously sends data to the AJLC monitoring system. It is essential that the conductivity ports in this probe are regularly cleaned to ensure the accurate data are collected.

Cleaning the conductivity probe:

- 1. Carefully disassemble the PVC pipe assembly to allow manipulation of probe.
- 2. Clean the outside of the probe with a soapy sponge.
- 3. Use the conductivity brush (in cardboard box inside DO maintenance toolbox) to clean the conductivity ports. Screw the brush into the conductivity port all the way, unscrew a few turns and then pull straight out.
- 4. Rinse the sensor with a hose and return to effluent sump.
- 5. Re-assemble the PVC pipe assembly that protects the sensor wire and put the lid back on the effluent sump.

⁶ This recipe is specific to Type I oxyguard probes that contain a zinc anode.

⁷ Crites, Ron and George Tchobanoglous 1998 Small and Decentralized Wastewater Management Systems. WCB/McGraw-Hill, p.47-48.

Checking and Recording Data on the Web Site

1. Checking Web Data

In addition to collecting data with hand-held probes, we also gather continuous data with sensors installed directly in various LM tanks. Data from these sensors is collected every minute, stored in a data logging computer and then transferred to a database on our server and displayed graphically on the AJLC website. When other operations tasks are complete, log into the computer in the mini-lab. (Username: envsadmin/Password: blue Check "workstation only").

Check DO and TSM data:

On the desktop, click on shortcut to DO_TSM data (<u>http://www.oberlin.edu/ajlc/systems_lm_3.html</u>)



Examine trends in dissolved oxygen concentration over the past two days, week, and month. Verify the following:

- 1. The date/time stamp at the top of the page is current.
- 2. All five tanks in which dissolved oxygen is measured (CA1, CA2, OA1, OA3, and Clarifier) are represented with lines.
- 3. That dissolved oxygen concentration is within the range given below for each tank:

CA1: 6-10 mg/L CA2: 6-10 mg/L OA1: 7-10 mg/L OA3: 7-10 mg/L Clarifier: 7-9 mg/L

Note: Average DO ranges for each tank in the Living Machine are high due to low biochemical oxygen demand. The AJLC Living Machine is significantly over-sized for the volume of wastewater produced by the AJLC (2470 gpd compared to 50-200 gpd average).

4. That daily TSM measurements are reflected by dips in dissolved oxygen at 10am, 4pm, 10pm and 4am (except in the clarifier).

If one probe (or more) is reading significantly lower or higher than the ranges given above, check for the following:

- 1. that the probe has been washed recently, and nothing is clogging the membrane's surface or the sides of the mixer
- 2. that the stir bar is spinning properly and is not worn out.
- 3. that the light indicator in the orange DO junction box is not brighter than others
- 4. that the motor is running smoothly (no evidence of leaks such as condensation behind glass plates, or damaged cable junction)

If the membrane is clogged, wash off the entire probe and motor thoroughly and replace in the tank. If no stir bar is present or it is worn out, replace it. Extra stir bars are stored in the DO probe toolbox.

Keep checking the web data to see if the DO climbs back to its regular levels. If this does not work, contact the TSM system student operators or John Petersen who will repair or replace the probe.

Check water flow:

Click on the desktop shortcut to flow data (<u>http://www.oberlin.edu/ajlc/systems_lm_2.html</u>). Examine flow trends for the last week and confirm that flow data reflect patterns of building usage. Look for spikes in usage during the week or when special events are occurring (parents weekend, orientation, commencement, AJLC events) and for drops or zero water use during breaks, summers, and weekends. This graph has aided in detection of water leaks in the building before. Please examine carefully and report anomalies to Cheryl Wolfe-Cragin or John Petersen.

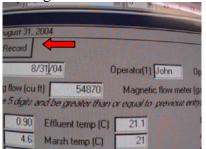
If these pages do not display graphs or display data that do not seem accurate or up to date email website coordinator.

2. Entering Operations Data into the Electronic Database

After all operations have been completed and the web data checked, click on the desktop shortcut named "LM log." This will open the Microsoft Access application and a window titled "Form. LM log."

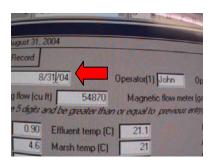
The log will open to the first entry in the database. Don't do anything to this form, as anything you do will overwrite old data!

1. Click on the box in the top left corner called "Add New Record." This will take you to a blank form where you can begin entering data.

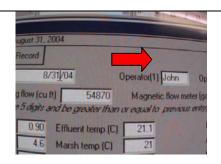


3. Enter the last names of the two operators that day. It does not matter which is entered first or second.

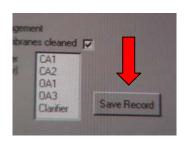
2. Enter the date in the form of mm/dd/year. Access will automatically change the format to include the day of the week.



4. Complete the rest of the form with the operations data you just collected. You must fill in all the spaces (other than the Water Quality Sampling information and the weekly and monthly responsibilities at the bottom) or Access will not let you save the form!



5. When you are finished, click on "Save Record" in the bottom right corner. The data you just entered will be saved into the database.





6. Exit Access and shut down the computer.



Management and Assessment of Organisms

Plant and Pest Management

Foliage maintenance and weighing cuttings:

A pruning shears should be used to trim plants with dead or infected leaves and those that are falling into walkways. When trimming back leaves, try to cut the stalk as close to the base as possible.



When all plants have been cut, separate them by species. A species display that shows their locations within each tank can be found on the wall of the minilab. Prepare the scale (hanging from the ceiling near the entrance) by turning it on, then hanging one of the orange plastic baskets (usually sitting outside the building near the CA tanks or under the table near OA1) from it. Press the [tare] button. This makes the balance's reading zero. Weigh each species' cuttings separately and record them on the log sheet. Once we know the dry weights of each individual species, we can go back through logsheets and determine how much dry-weight of biomass we really harvested (as opposed to biomass + water weight). When finished, take all the cuttings out to the compost pile behind the permaculture garden by Harkness.

Pest management:

The main forms of pest management employed by the operations crew are: regular spraying of plants with the water hose, spraying of infected leaves with a garlic spray, manually killing the pests.

Spider mites and aphids have been the prime plant pests in the Living Machine since the fall of 2000. Therefore our pest management regime has been geared mainly towards these organisms. However, slugs and midge flies have also been problems.

Spider mites are almost too small to see but signs of their presence include a web-like material covering the leaves and stems or leaves that are becoming yellow and dying. Eliminating spider mites is extremely difficult. To rid the leaves of current mites, directly wipe leaves with a wet sponge. After operations every day, all the plants should be sprayed with the hose, as our research has found that spider mites have more difficulty reproducing in wet conditions. Affected leaves can be treated with garlic spray in the case of a bad infestation.

Aphids, though larger and more visible, are just as difficult to remove. Spraying the infested plants with a high-pressure hose can physically remove some of them, but ultimately manually wiping down

the plant may also be necessary. As with spider mites, spraying all the plants after operations will make conditions less conducive to aphid reproduction. In the event of a particularly bad infestation, spraying the affected leaves with the garlic spray has been shown to be effective.

Slugs are harder to control because they're active mainly at night. If you happen to be in the LM during the dark hours, feel free to drop some salt directly onto slugs and watch them shrivel like the disgusting creatures they are. Salt can be found on the "Pest Management" shelf in the plastic cabinet. Plastic cups full of cheap beer have also been effective in attracting and drowning slugs.

Midges look like mosquitoes, but they don't bite. In the late spring and early summer of 2000 an incredible midge infestation occurred. Clouds of thousands turned patches of the white LM walls black, they plagued visitors and operators, and eluded our attempts to spray them with water and prevent them from reproducing. Ladybugs were introduced into the LM because they're excellent natural predators of many insect pests, but these died quickly and were ineffective. Then, in late June 2000, spiders began entering the LM through window and door cracks from the outside. Within a week the midge population was under control, and a boom and bust predator-prey cycle ensued the midges were gone. From this experience, we learned that in some cases it's best to let natural processes take over when all our human attempts to select an ecological community in the LM have failed.

In addition to spraying the plants regularly, cutting severely infested leaves is advisable to prevent the spreading of the pests to other plants or other tanks. Remove the leaves from the LM greenhouse as soon as possible after cutting and weighing. Chop cuttings into smaller pieces and put them in composting bin #1 (western-most bin)

A list of LM plants as well as a map of the open aerobic tanks and what is planted in each of them can be found at the end of this document.

Other Maintenance

1. Cleaning Tasks

Ensuring that the Living Machine remains clean and organized is crucial to operator and visitor safety. Specific cleaning tasks are assigned to each day. Cleaning supplies can be found both in and beside the cabinet. Operators must wear gloves and safety glasses while cleaning in the Living Machine.

MondayClean outside and rim of OA1 & 2TuesdayWash both sides of all greenhouse door glass AND handlesWednesdayWash outside and rim of OA3 & ClarifierThursdayTake trash to dumpster behind Talcott and replace bagFridayOrganize and wash operations counters and cabinetsSaturdayScrubClarifier spillway (both compartments), airlift pump standpipes and all windowsills

Glass doors and windows should be cleaned using Dr. Bronner's soap or vinegar and a sponge and rinsed with water. A scrub brush on a telescoping arm is next to the cabinet for high areas (Do not ever stand on top of tables or ladders to reach anything in the LM!). The small or large squeegee should be used to remove excess water from glass.

All other cleaning tasks LM can be completed with a few drops of mild soap like Dr. Bronners in a bucket of water and a sponge. 70% ethanol can be used to sterilize counter tops, equipment housings, and door handles. TSM probes pulled from tanks for renovation should be transported to the lab in a garbage bag and cleaned with a 5% bleach solution.

2. Preparation/Stocking of Sample Collection Materials

Lab Glassware and Equipment Cleaning: Tues/Thurs/Sat

Your job is to make sure the acid bath does not get over-crowded. It is <u>not</u> your responsibility to keep track of supplies not set into the acid bath. Students doing water quality sampling each day are responsible for initially washing their materials and placing them in the acid bath.

- 1. Put away all dry lab materials on racks.
- 2. Remove all Living Machine sample containers, filter apparatus for BOD and fecal coliform, and other processing devices from the acid bath.
- 3. Follow steps 7-9 below.

Stock Sample Containers in the Living Machine: Sat or Sun

Stock the Rubbermaid container in the Living Machine labeled "water quality sampling" with the following clean, acid-washed and labeled bottles by Sunday evening each weekend. Acid washed bottles are located under the dishwashing sink in the lab, on shelf to right of sink, and on top of refrigerator.

	Label Tape	Monday	Wednesday	Friday
Post-UV	Yellow tape	3-125ml	3-125ml	3-125 ml
Effluent	Green tape	3-125 ml	3-125 ml	3-125 ml
		2-1L	2-1L	2-1L
AN1	Red tape	1-125 ml	None	None
		1-0.5L		
	Total bottles	10	8	8

Washing glassware

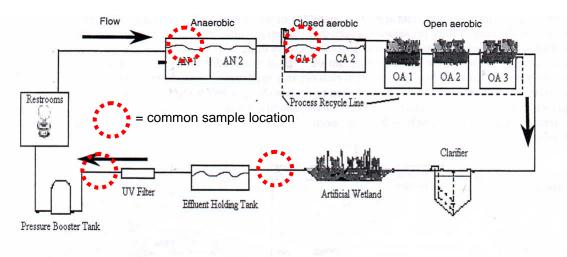
Procedures for washing glassware are described in Methods for Analyzing Aquatic Ecosystems.

Sampling methods:

The methods that lab assistants should to collect, process, store and analyze water samples from the Living Machine are described in Methods for Analyzing Aquatic Ecosystems

Sampling locations:

Living Machine lab assistants collect, filter and process water samples from influent (AN1) or CA1 and effluent (marsh sump) for bacterial, organic matter, and nutrient concentrations. Less frequent samples are collected from AN1, CA1, OA1, OA3, Clarifier, Effluent. Do not collect samples from the Living Machine unless you have been trained by an experienced operator, faculty or staff person.



Sampling Schedule (Fall '06):

	Nutrients/BOD	Fecal Coliform
Monday	1.5L Effluent, 0.5 L CA1	3-125 ml Post-UV
-		3-125 ml Effluent
		1-125 ml CA1
Wednesday	1.5L Effluent	3-125 ml Post-UV
_		3-125 ml Effluent
Friday	1.5L Effluent	3-125 ml Post-UV
_		3-125 ml Effluent

Aquatic Microorganisms of the Living Machine⁸

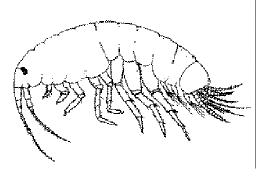
Scuds or Side swimmers Kingdom: Animalia, Phylum: Arthropoda, Class: Crustacea, Order: Amphipoda

Description/ Taxonomy: Scuds or Side swimmers are one of the orders of the subphylum Crustacea, and class Malacostraca. Scuds range from 5-20 mm (ours seem to be on the smaller end of this range) and are light gray or cream colored. Some seem to have a darker black stripe on their back/ head. The body is strongly flattened from side to side, and there are two pairs of antenna on the head. They often roll over on their side as they swim, hence the name sideswimmer. Scuds breathe through gills. Refer to some of the cited works for a more technical anatomical description.

Location: In the Living Machine scuds are found in the OA tanks, near the surface of the water swimming around root masses. They are often inside the TSM probe in OA1, and can also easily be seen swimming around in the tanks. For closer inspection, use the dissecting microscope in the lab.

What do they do? Scuds are omnivores, and their most common food is either fine or coarse detritus. They also feed on thin films of algae, fungi and bacteria on submerged plants and objects. Since scuds consume so much detritus and organic matter they are important in ecosystems for removal and breakdown of waste. In favorable habitats, numbers have been known to exceed 10,000 per square meter.

Indicator Status: Scuds are restricted to cool, clean, well oxygenated waters.





Left: Basic scud morphology. Right: Scud from OA2, 40x magnification, dark field.

⁸ Kevin Kralik '04 Living Machine Summer Intern

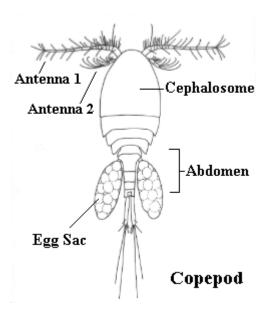
Aquatic Mandibulates, Class: Crustacea, Order: Copepoda Copepods

Description/ Taxonomy: Copepods are tiny multi-celled animals barely visible to the naked eye. American species range from .3 to 3.2 mm; the ones observed in the Living Machine are probably about one millimeter. They are visible with the naked eye, and examination under the dissecting scope allows for detailed inspection. Copepods are very distinctive, and the male can even be distinguished from the female by the presence of egg sacs attached to the abdominal segments. Copepods are the most numerous animals not only in the plankton but also in the world; the individuals of a single genus (Calanus) are thought to outnumber all other animals put together.

Location: Copepods have been found in OA1. They are most likely present in all of the OA tanks and possibly the clarifier.

What do they do? Bacteria, algae and protozoans are all food for copepods.

Indicator Status: Copepods can withstand some oxygen deprivation; probably not a good indicator species.



Phylum: Gastrotricha

Description/ Taxonomy: Gastrotrichs found in the LM will belong to the order Chaetonotoidea, since this order is mainly freshwater, while the other order in the phylum is strictly marine. There are about 400 species in this phylum. Gastrotrichs are fairly large at about 100 to 300 microns. They are easily detectable at 40x magnification, although for closer inspection 200x or 400x is useful. They do move around fairly quickly, however, so too high a magnification makes them difficult to keep in view. Gastrotrichs are pretty distinctive; when one appears it should be easy to recognize. Locomotion is generated by beating cilia on the ventral surfaces. There are no specialized respiratory or circulatory organs in Gastrotrichs, but they do have simple digestive, nervous, and reproductive structures as well as simple muscle and protonephridial (primitive kidney like) systems.

Location: Gastrotrichs are common in fairly shallow, still or very slow moving bodies of water where there is abundant detritus, so it makes sense that they are common in the LM. Most don't venture very far from the substrate, and they are rarely found swimming in open water. They seem to be most common in the LM in the clarifier. Scraping some of the algae off the sides of the tanks or pipes will yield small algae particles in the sample. Under the microscope (probably 40x, or the 4x objective) scan these little algae clumps for Gastrotrichs swimming around. They are very active and almost always moving.

Diet/ Predators: Diet consists of bacteria, algae, organic detritus, and small protozoa. Gastrotrichs forage about the substrate for their food, and they can often be seen feeding under the microscope. Cilia beating around their anterior mouth opening help to bring in food. Gastrotrichs may be eaten by insects and crustaceans, amoeboid protozoans, hydras, and possibly even nematodes.

Indicator Status: As stated above, these organisms occur in habitats with decaying organic matter. Since they can be found in areas with high amounts of decay, species have been collected when dissolved oxygen is less than 1 ppm (mg/L). Lower levels of DO for Gastrotrichs are not known, but they can most likely with stand temporary anaerobiosis. Generally they are found when dissolved oxygen is high and system is extremely stable.



Gastrotrich 200x magnification, from clarifier

Phylum Rotifera:

Description/ Taxonomy: Microscopic, wormlike or spherical pseudocoelomate animals, "wheel animalcules". An estimated 1,500-2,000 species of rotifers range in size from about 100-500 microns. They have three distinct body regions- the head, trunk and foot. Locomotion is accomplished by

cilia, the beating of which suggests a wheel. There are typically different varieties of rotifers for every stage of the activated sludge process within any one wastewater treatment system.

Location: Rotifers have been found in the OA tanks. The clarifier may also have rotifer populations. They are typically found in stages with at least 3 mg/L of oxygen.

Diet/ Predators: Depending on their location in the treatment system, rotifers may feed on algae and phytoplankton, bacteria, or flocs

Indicator Status: Rotifers are important organisms in wastewater treatment. They stimulate microfloral activity and decomposition, help recycle minerals and enhance oxygen penetration. Rotifers help to enhance floc formation by snipping off particles of floc which create new surfaces for floc formation and also allows oxygen to penetrate the floc. The same is true for slime layers. Floc formation is also enhanced as rotifers feed on non floc forming bacteria. Finally, in aerobic processors Rotifers help to reduce BOD by consuming organic matter. With greater stabilization of organic material and higher effluent quality, higher and higher life forms appear. Rotifers represent the next stage of effluent quality (next higher life form) beyond stalked ciliates.



Kingdom Protista, Phylum Protozoa:

Description/ Taxonomy: There are so many varieties of protozoa that identification can become very difficult. The nomenclature system is rather confused and complicated. Many levels of classification can only be determined through genetic studies, but some can be distinguished to a certain degree under the microscope. Protozoa are single celled organisms that have organelles and nuclei; they are

more complicated than bacteria which have no organelles or nucleus that contains genetic material. Protozoa range in size from a few microns (1 micron is 1/1000 of a millimeter) to several hundred microns. Some of these larger protozoa can even be much larger than some small multicellular aquatic invertebrates. Here is a rough idea of the protozoa most likely to be encountered in the LM, and their relation to one another:

Phylum Protozoa:

Subphylum Sarcomastigophora Superclass Mastigophora (Flagellates) Subphylum Ciliophora

Class Ciliata

*Subclass Holotricha: Simple, uniform ciliation around the whole organism. *Subclass Peritrichia: Non uniform cilia- cilia only around mouth for example.

There are many more classes and superclasses, etc, but this is the basic idea for things you are likely to see in the LM.

Location: Many protozoa are attached to clumps of organic matter, and others generally stay close to the substrate because it affords food and protection. The best way to find protozoans is to scrape any kind of slime, ooze, etc from the walls or plants of the tank. They are also present in floc, such as the sludge from the bottom of the clarifier. Plant roots are often covered with a thin film of organic material that can be a good source of protozoans.

Diet/ Predators: Some protozoans feed on soluble organic matter, some are bacteriovores and some are carnivores (feed on other protozoa, usually ciliates).

Indicator Status/ Significance: Indicator status of protozoa varies between different groups. In general though, protozoa of various kinds are invaluable in wastewater treatment systems for the following reasons: **Bacteria Removal:** Many protozoans feed on bacteria, and in the aerobic stages of treatment protozoans are essential to bacterial removal- systems without protozoans have much poorer effluent quality. In fact, bacterial removal has been shown to improve from 50% with no protozoans to 95% with ciliated protozoa. **Growth Simulation of Bacteria:** Protozoa grazing on bacteria keeps the bacterial population healthy by removing old and diseased bacteria and encouraging more reproduction.

Genus *Coleps:* Carnivores that do not use currents created by cilia for feeding. Found in OA tanks and clarifier.



Coleps 400x magnification from clarifier

Genus Stentor: Present with the development of a mature sludge. Found in the clarifier.



Stentor 40x magnification, Dark Field, from the clarifier sample



Stentor 200x magnification, from the clarifier

Genus *Vorticella(?)* : Indicative of a stable, well operating plant with mature sludge. A conflicting source reports that they can be indicators of good effluent quality (oligosaprobic), intermediate quality (mesosaprobic) or inferior (polysaprobic) depending on the species. Found in the clarifier.



Vorticella 200x magnification, from clarifier sludge

Free Swimming Ciliates: Generally more free swimming ciliates means better settling of floc and higher quality sludge. They consume huge quantities of bacteria which is essential to the bacterial removal stages. Found in clarifier, OA and CA tanks.



Ciliate from the clarifier, 400x magnification