Cup Horn Manual

Model #431C2

Qsonica, LLC.
53 Church Hill Rd, Newtown, CT 06470 USA
Phone: 203.426.0101 or 877.338.9636
Fax: 203.426.7026
E-mail: info@sonicator.com
www.sonicator.com

Rev. A. 7-29

Overview

Cup horns offer indirect sonication and function as high intensity ultrasonic water baths. Multiple samples can be processed in sealed tubes or vials eliminating aerosols and cross contamination. Cup Horns are ideal for sterile or pathogenic sample processing and enable multiple tubes to be processed at one time.

The horn is mounted within an acrylic cup and the cup is filled with water. Sample tubes are placed in a rack just above the horn. Cavitation is produced in the water, processing the samples within the tubes or vials. Coolant ports are on each side of the cup for circulation of cold water to keep samples from overheating. A microtube rack and holder are included with each Cup Horn.

The Sound Enclosure (#432B2) is highly recommended for all Cup Horn users. In addition to reducing sonication noise to safe levels, it securely supports the Cup Horn in the proper position. The Sound Enclosure features ports on either side to allow coolant tubing to pass from the Cup Horn to a water source or pump system outside the box. Please contact Qsonica for information on the Recirculating Chiller (Part# 4900) option.

Q700, Sound Enclosure, Cup Horn & Chiller

#431C2

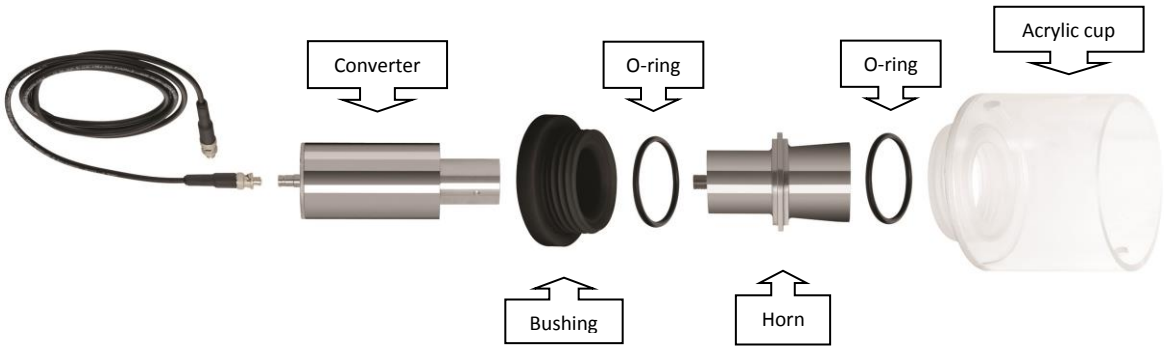
The cup horn includes a sample rack (part# 440) that can accommodate 8 polystyrene tubes (1.5ml). The unit is shipped with a set of polystyrene tubes which are proven to transmit ultrasonic energy better than standard polypropylene tubes. Polystyrene tubes have large caps so they require this specific tube rack.

Note: Superior results are achieved by using polystyrene tubes.

Polypropylene tubes work well for shearing genomic DNA or other "easy" applications. *(Note: A special sample rack is **required** for polypropylene tubes (part #451)).* Difficult to lyse samples such as yeast and tissues or chromatin fixed with formaldehyde will require the polystyrene tubes.

Other sample vessels (i.e. beakers, 15ml centrifuge tubes, etc.) can be processed in a cup horn. The user must determine an appropriate way to support or float the sample vessel in the cup horn reservoir. See *Sample tube/vessel Information* section below for additional information.

Parts List



Quantity	Description
1	Bushing
2	O-rings
1	Horn (titanium)
1	Acrylic cup
1	Tube rack
1	Tube rack holder (bridge)
	Converter and cable are included with the purchase of the Sonicator system

Installation

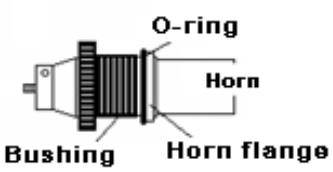
1. Ensure that the ultrasonic processor is OFF and unplugged. If you have a standard probe on your sonicator, follow #2. If not, skip to #3.

2. If a probe is attached, using the spanner wrenches provided with the power supply, remove the probe from the converter.

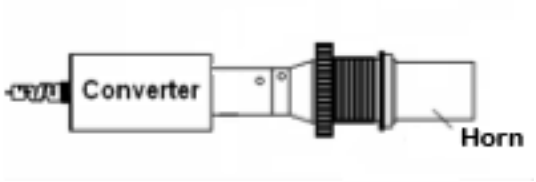

REMOVAL


TIGHTENING

3. The Cup horn will be shipped with the cup attached to the titanium horn. In order to tighten the convertor to the cup horn it is recommended to remove the clear acrylic cup from the black plastic bushing. Set the clear cup and one o-ring to the side. Place one O-ring and bushing on the side of probe with threaded stud.

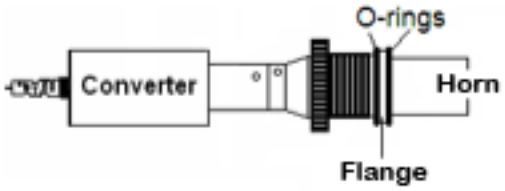


4. Hand tighten the cup horn to the convertor. Then place the convertor and horn assembly horn side down on a bench top. Use wrench set provided with your Sonicator to tighten the cup horn onto the converter as show in the figure below. The cup horn will malfunction if not tightened well.

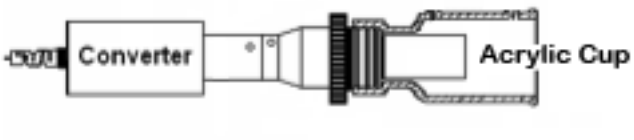


Attaching Cup horn to converter

5. Place the other O-ring on the other side of the probes' flange. Ensure the two o-rings and black plastic nut are seated properly on the titanium horn. *A little o-ring lubricant may be used to ensure a proper seal (and prevent o-rings from drying out) but it is not required.*



6. Place the plastic cup over the titanium horn and secure in place by screwing the bushing into the threaded portion of the plastic cup. Do not over-tighten.



7. The cup horn is supplied with both plugs and hose barb fittings (which are compatible with ¼" ID tubing). The hose barb fittings must be used if cold water will be recirculated through the cup horn (recommended method) to help maintain sample temperature. Otherwise, plug fittings can be used to hold water in the reservoir. Attach the appropriate plug or hose barb fittings. It is highly recommended to use Teflon tape on

all fittings to ensure a water tight seal. It is not necessary to screw the fittings all the way into the cup horn. Just enough to hold water is adequate.

8. Check for leaks: Fill the cup horn with DI water to just below the hole of the lower outlet port to ensure that the o-rings, cup and nut are assembled properly and are not leaking water. Drain all the water from the cup reservoir after this step. *Be careful not to allow water to drip into the Converter as it is an electrical part.*

9. Mount the converter/cup horn assembly in the sound enclosure (part #432B2). This accessory will properly support the weight of the filled cup horn during sonication. Note: *The cup horn can generate over 100db. QSonica recommends using the Sound Enclosure accessory to reduce the noise of sonication down to safe comfortable working levels.*

   The convertor cable should be inserted into the bottom of the enclosure and connected to the converter. Slowly lower the convertor/horn assembly into the convertor collar of the stand or mount in the converter holder of the enclosure. Do not allow the cable to kink or bend inside of the collar.

10. If temperature monitoring is required (and your sonicator model has that capability), attach the temperature monitoring thermocouple (part #4103) to the Sonicator generator. Place the end of the thermocouple wire into the cup. Once the cup is filled with water, ensure the end of the wire remains submerged. The thermocouple can also be placed in a blank sample tube of buffer if preferred.

Filling the cup horn with water:

During operation of the cup horn, the ultrasonic energy transmitted into the water will cause the temperature to rise. If the sample is temperature sensitive, you must take steps to circulate cold water through the cup. Qsonica offers a re-circulating chiller (#4900) specifically designed for the cup horn.

Static Reservoir - *For brief processing times (15 minutes or less)where chilling the water is not necessary*

1. Ensure the plug fittings provided with the cup horn are installed into both ports on the cup.

2. Have a source of chilled DI water to fill the cup horn.

3. Place the holder and attached sample tube rack with samples into the cup.

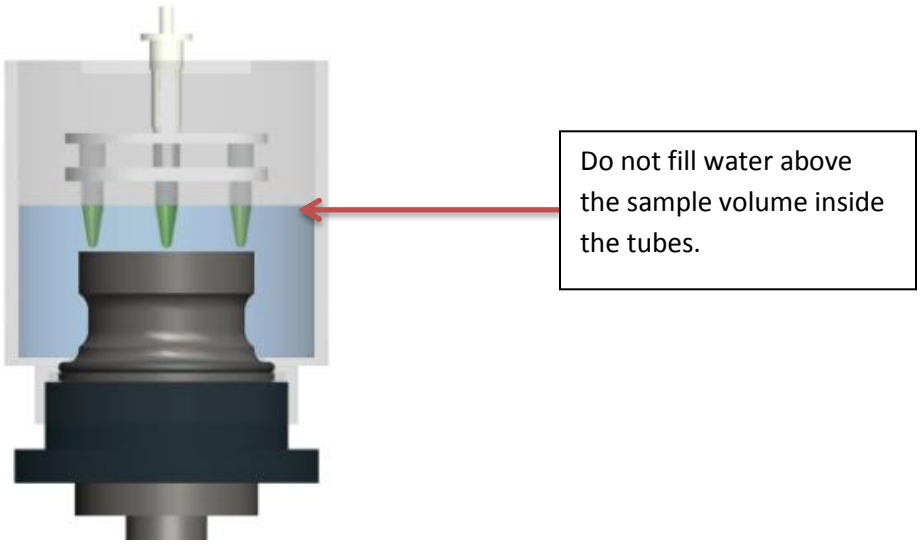
4. Slowly add water along the side of the cup until the water level is equal to the sample liquid level inside of the sample tube (see the picture below). Once the appropriate water level is determined for your sample volume and sample tubes, laboratory tape can be used to mark the water fill level on the outside of the cup. *The water level must remain constant to prevent variability when processing samples.*

5. If sonication is brief, the heat generated in the cup will not affect the sample. If the water temperature rises during sonication, pour it out (or use a pipette) and refill the cup with cold water to the same volume as previously used. *Note: To further lower the temperature, crushed ice can be used to help cool the water in the reservoir. If using ice the water level must remain constant to prevent variability when processing samples. In addition, ice should only be placed at the periphery of the sample tubes and never under the tubes which would block the transmission of ultrasonic energy.*

6. If you have difficulty optimizing or achieving reproducible results see the figure below and adjust the water level accordingly. Samples may benefit from matching the water level to the liquid sample inside the tubes.

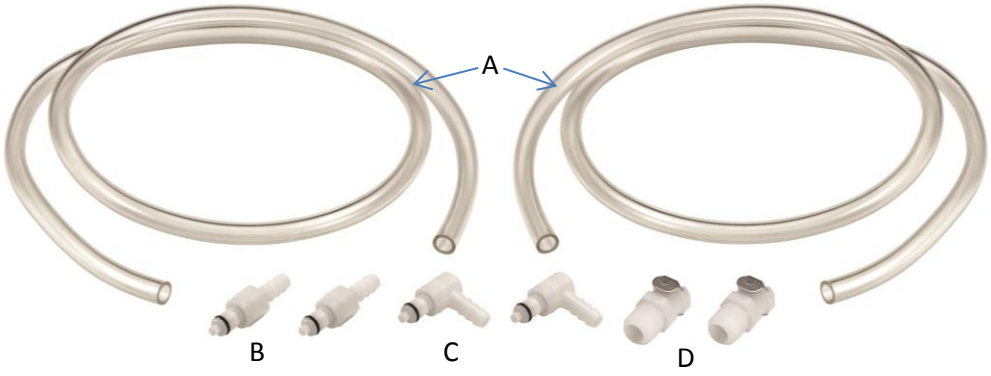
Water Level

The water level inside any type of Cup Horn **MUST** be equal to the liquid level inside the sample tubes.



Recirculating Chiller - *For constant flow cooling and required for extended sonication times (Recommended)*

1. Obtain part #4910, (Tubing and Connector set) as shown below. The tubing and connector set comes with tubing (A), two male straight hose barb connectors (B); two male right angle hose barb connectors (C) and two female connectors(D). *Note: The tubing (A) may come in one long piece and should be cut in half before it it is installed.*



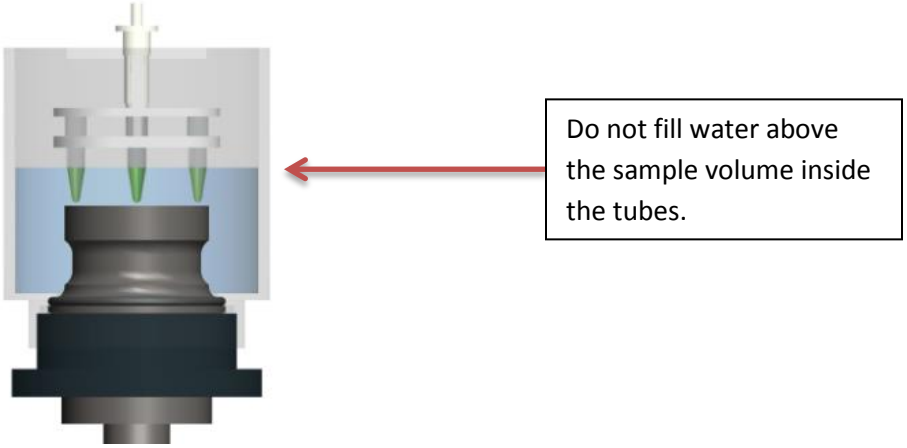
2. Attach the female fittings (D) to the cup horn in the inlet and outlet ports on the acrylic cup.

3. Attach one right angle male fitting (C) to one end of each piece of tubing and one male fitting (B) to the other end of each piece of tubing. Note: the tubing length can be shortened if necessary.
If you are using a different recirculating chiller or cooling method, ensure both ports on the cup horn are fitted with hose-barb adapters provided with the cup horn. Connect the cup horn to the alternate cooling source as necessary.

4. Insert one right angle male fitting to the inlet on the cup horn and the male fitting on the other end of the tubing to the coolant supply on the chiller. Repeat for the outlet and coolant return. Refer to the photo below:

5. When using the Sound Enclosure, there are capped ports on either side. Remove the caps and pass the tubing through each port before attaching the connectors. You have the option of using the ports on either side of the enclosure.

6. Using 4°C DI water, slowly add water along the side of the cup until the water level is equal to the sample liquid level inside of the sample tube (see the image below). Once the appropriate water level is determined for your sample volume and sample tubes, laboratory tape can be used to mark the water fill level on the outside of the cup. *The water level must remain constant to prevent variability when processing samples.*



7. Turn the chiller on and set the desired temperature with the key pad. The temperature can be set as low as 4°C. *Note: Do not operate the chiller without water in the cup.* Begin to recirculate cool water through the cup horn reservoir. As the tubing fills the water level in the cup will drop. Add additional water with a pipet if necessary to ensure that the water level is the same as the sample liquid level in the tubes as indicated above.

8. Allow the Chiller to recirculate the water through cup horn until a desired temperature is reached before beginning sonication. To greatly reduce set up time, store 2L of chilled DI water in the refridgerator for startup.

9. Note if your samples are temperature sensitive it is important to monitor the water temperature in the cup reservoir and not to rely solely on the temperature read out on the chiller. The LED display on the chiller shows the temperature of the water as it leaves the chiller and returns to the inlet of the cup horn (coolant supply). The actual temperature in the cup horn during sonication may be a few degrees higher.

Choosing Sample Tubes and Racks

It is important to choose the best type of sample tube to match your specific application. When using 1.5ml tubes, Qsonica recommends tubes made of polystyrene. These tubes work much more efficiently than standard polypropylene or Eppendorf tubes. *We do not recommend using standard polypropylene tubes for lysing yeast, shearing chromatin or other difficult to process samples.* Standard polypropylene should only be used for applications that require low sonication intensity.

Note: For most applications, processing samples over 1ml are not recommended.
Note: When used with the appropriate tubes, all racks hold tubes approximately 2mm above the ultrasonic horn.

The following tube racks are available. Note that specific types of tubes are recommended for each rack.

#440 – 8 tube holder (***Included with #431C2 Cup Horn***)

Made for use with Evergreen Scientific polystyrene 1.5ml tubes with caps. www.evergreenscientific.com; Part # 214-3721-010 300-2911-020

Recommended for difficult samples when using 1.5ml tubes.

#451 – 8 Tube holder

Made for use with standard 1.5ml Polypropylene tubes (Eppendorf, etc..)

Note: Polypropylene tubes do not transmit energy as well as polystyrene.

#449 – 12 tube holder

Made for use with Brandtech 500ul PCR sample tubes
www.brandtech.com Part #781310 (thin wall)

Thin walled PCR tubes work well for most applications.

#445 – 24 tube holder

Made for use with Brandtech 200ul PCR sample tubes
www.brandtech.com Part #781305 (thin wall)

Thin walled PCR tubes work well for most applications.

Note: Customized sample tube racks are available. Please contact us for more information.

Other Sample tube/vessel Information

Most laboratory grade plastic or borosilicate glass vessels that will fit into the cup are suitable for sonication. Polystyrene and polycarbonate are better transmitters of acoustic energy and are recommended. 1.5ml polystyrene sample tubes from Evergreen Scientific *(Evergreen Scientific, www.evergreenscientific.com; Sample tube part #214-3721-010, Cap part #300-2911-020)* are specifically recommended for use with our cup horns. Polypropylene and polyethylene are softer and may be less effective, however they are the most readily available and the majority of users have no problem optimizing sonication protocols with them. Glassware must be free of scratches or it may crack. Metal sample vessels can be used as well.

Ultrasonic energy radiates directly upward from the vibrating surface of the ultrasonic horn and through the sample vessel, while transmission through the side is negligible. For this reason, flat-bottomed vessels are best but round bottomed vessels work well for most applications. Vessels should have thin, uniform bases, without any heavy edges or centers. Conical tubes can be used, however, sonication protocols may require longer sonication time. If using your own sample vessel, maintain a small clearance around the vessel to allow adequate coolant flow.

Cup Horn set up for various size sample vessels:

The cup horn has been optimized and equipped with a sample tube rack for processing 1.5-2mL polystyrene microtubes. The cup horn can be used with other sample vessels as well. Tube racks for 500 and 200ul thin walled PCR tubes are also available. Smaller tubes are recommended when processing smaller samples.

The following are examples of how to support various sample vessels:

- A sample vessel like a 5ml or 15ml Falcon tube can float horizontally in the cup horn reservoir.
- Three prong clamps can be obtained from a laboratory equipment dealer and used with a laboratory support stand to hold samples like beakers or Falcon tubes (vertically) in place. Do not allow beakers or vessels to touch the titanium horn.

Operation

Key points to note:

- Keep cold DI water in a refrigerator/cold room so it's always available when needed.
- Keeping the water level constant is important to ensure consistent results.
- Polypropylene tubes work well for many applications but polystyrene or polycarbonate tubes transmit the ultrasonic energy more effectively. Difficult to process samples may benefit from using polystyrene tubes.
- Make sure that you have the appropriate sample tube rack for your sample tube type.
- A chiller is recommended instead of ice to cool the system.
- Use the pulse mode to aid in cooling the system. The longer the pulse off duration, the cooler the system will remain.
- If you must use ice, do not allow it to flow under the sample tubes. Only add ice to the periphery of the cup to prevent it from obstructing the ultrasonic energy.
- Appropriate amplitude and time settings must be determined by empirical testing!

Once your samples are in the cup horn and the cup horn is filled with water to the appropriate level operate/program the sonicator as described in the Sonicator manual.

Optimization

The first time you use the Cup Horn, it is important to run the system and observe the sonication field with water only and no samples. Notice the tiny bubbles traveling through the liquid, as this is visual evidence of the ultrasonic energy. The swirling action above the Horn may resemble a miniature tornado. As you increase amplitude the intensity of the sonication increases and travels through the water column.

Cup horns produce a lower intensity than direct sonication methods (standard probes and microtips). Direct sonication settings cannot be used with cup horns. Although sonication protocols will be application dependent, many Cup Horn protocols require higher amplitude settings (i.e. 65-100%) and longer duration times when compared to probe sonication. It is not uncommon for difficult cell types to require 30-60 minutes of sonication time in a Cup Horn.

For maximum transmission of acoustic energy into the sample vessel:

- Consider using a polystyrene or glass sample vessel. The more rigid the sample vessel material the more efficient the transmission of ultrasonic energy.
- De-gassed liquids are more efficient at transmitting ultrasonic energy. Filling the cup horn reservoir with DI water and then turning on sonication for 60 seconds before adding your samples will ensure that the water in the reservoir is degassed.

Optimizing Sonication Protocols

In order to optimize a sonication protocol you must first minimize both sample and set up variables. Begin with one sample volume (i.e. 300ul) and one sample concentration (i.e. 5ug of DNA or 20 million cells or 1ug of excised tissue, etc.). Have at least 9 of the same sample available to begin optimization (this is a minimum suggestion, test additional samples if needed). Load samples and set water level properly. Program the unit at the following amplitude and time settings :

- 50% - 1 sample for 10, 15 and 20 minutes total sonication ON time
- 75% - 1 sample for 10, 15 and 20 minutes total sonication ON time
- 100% - 1 sample for 10, 15 and 20 minutes total sonication ON time

Note: Lower amplitude can be tested if your application only requires low intensity.

Pulse Mode
In order to control temperature, program the sonicator to **pulse** On/Off as needed. For example, 10 seconds ON and 20 seconds OFF is a common starting point. Monitor the water temperature and adust the pulse mode to control temperature as needed.

Results
Examine and compare the results to determine lysis or shearing efficiency at each intensity and time setting. Complete additional rounds of testing if necessary to determine an appropriate sonication time to achieve desired results. Remember to minimize any changes to sample variables (i.e. concentration, volume, etc.) while testing.

Note: Once an amplitude setting that best processes the sample is determined, adjust the total sonication time until a desired result is achieved. Once an optimal sonication intensity setting is determined for a specific sample, changes to a sample variable (i.e. volume or concentration) may require adjustment to the total sonication time to achieve the same results.

Examples of sample and preparation variables that will have an effect on sonication protocols and may require adjustments to a sonication protocol can include (but are not limited to):

- Changes in total sample volume
- Sample concentration
- Temperature
- Crosslinking fixation time (if you are working with ChIP samples)
- Cell line
- Cell origin (tissue or culture)

Examples of sonication set up variables that will have an effect on sonication protocols and may require adjustments to a sonication protocol can include (but are not limited to):

- Type of sample tube used
- Temperature of water reservoir
- Distance samples tubes are from the ultrasonic horn

Maintenance

It is recommended that the cup horn be disassembled and cleaned periodically with a mild detergent. The cup is made of acrylic, therefore solvents cannot be used within the cup or for cleaning purposes.

The cup horn should always be tightened properly to the converter. With normal use the cup horn can loosen over time due to ultrasonic vibration. Therefore maintenance schedules will depend on frequency of use. The Cup Horn should be inspected monthly, cleaned and re-tightened as needed.

The cup horn should not be stored overnight with water inside the reservoir. Keeping the unit dry will inhibit the growth of mold.

If recirculating water through the horn for cooling, the tubing connected to the outlet port may be used to drain the water in the reservoir. Any water left in the base of the cup horn should be removed with a pipet or soaked up with a paper towel. Water in the tubing and chiller should be drained as well.

Cleaning Procedure

1. When cleaning the cup horn always remove it from the convertor.

    a. Drain all water from the cup reservoir.
    b. If hose barb fittings and tubing were used to re-circulate water through the reservoir, ensure that they are drained of water and the tubing detached from the cup.
    c. Unscrew the clear plastic cup from the black nut and set aside.
    d. Use the pin spanner wrenches provided with the Sonicator System to loosen the cup horn from the converter as show in the figure below.
    e. Once the cup horn is loose it can be unscrewed by hand from the converter. *Be sure to place the converter on a surface that will not allow it to roll off a bench or table.*

Removing the cup horn from the converter

2. Clean all parts with a mild detergent and warm water. The titanium cup horn can be autoclaved if necessary. *Do not clean plastic parts with solvents or abrasives (may cause the acrylic to crack).*

3. Clean the threaded mating surfaces on the horn stud and convertor with a cotton swab and alcohol.

4. Allow parts to dry. Do not allow any liquid to drip into the convertor opening when cleaning the threading. *All surfaces must be dry before assembly and tightening.*

5. Inspect all parts of the Sonicator system and cup horn for damage before re-assembling the cup horn. Pay particular attention to the threading on the cup horn stud and converter (it should not be scratched), the o-ring should not be dried out or cracked, the cup horn/fittings should not leak water into the converter, if using tubing to re-circulate water it must be replaced if dirty or cracked.

6. Reattach and tighten the cup horn assembly as directed in the installation instructions.

Note: The horn is a tuned resonant body of titanium alloy. Do not attempt to resurface the horn if it shows erosion after extended use. Wear on the face of the horn is normal over time and is expected. All ultrasonic horns are considered consumable parts that will wear over time and require replacement. The useful life of a cup horn is dependent upon the intensity setting used and the frequency of use. It will vary between users and applications.

If you suspect that your horn is worn and requires replacement, please contact Customer Service.

Daily: Drain the system (Cup horn reservoir, tubing and chiller) at the end of each day. This will prevent the water in the system from becoming stagnant and growing bacteria/mold.

If contamination does occur please take one or more of the following steps to remedy the issue:
1. Flush the system with fresh water and drain. Repeat.
2. Use Clear Bath® (http://www.spectrumlabs.com/labware/ClearBath.html) to treat and keep water in the system clean.
3. Drain the Cup, remove from the sound enclosure and disconnect from the converter. Remove the cup from the horn and wash with a mild detergent. Rinse and allow parts to dry completely. Reassemble horn and then tighten it to the converter with the wrench set provided. (see the section below on Cup Horn assembly for assistance).
4. Inspect tubing and replace when necessary.

As needed:
1. Horn/converter must be properly tightened. If the horn is not tightly connected to the converter you may see an overload error message, a fluctuation in wattage reading or a change in the noise level. Please refer to instructions in the Cup horn assembly section below. Note: A loose horn may cause damage to the generator circuitry or parts of the converter and horn.
2. Check horn for signs of wear with normal usage. Using a severely worn horn can damage internal generator components. Contact Qsonica if the horn's surface shows signs of pitting or excessive wear.
3. Replace tubing as needed.

Chiller:
Refer to the Chiller manual for additional maintenance information for this device or the filter that accompanies it.

Troubleshooting

Your Ultrasonic Processor was designed to provide you with years of safe and dependable service. Nevertheless, because of component failure or improper usage, the possibility does exist that it might not perform as it should, shut down or stop working all together. The most probable causes for malfunction are listed below and should be investigated.

- The horn is not tightened properly with the wrenches provided. Remove and re-tighten.
- A connector or cable is damaged.
- The unit was plugged into an electrical outlet that provides a different voltage from that required. See *Electrical Requirements.*
- The Horn being operated is worn past its useful life.
- A fuse(s) has failed.

OVERLOAD CONDITION

If the Ultrasonic Processor stops working, and an OVERLOAD indication is displayed on the screen, check for possible causes as outlined in the above paragraph. Then press the **OFF** key to switch the unit off, and the **ON** key to switch the unit back on to restart the equipment.

If the problem persists after inspecting all of these, please contact Customer Service for additional assistance or to replace a worn microtip or damaged part.