
USER'S GUIDE

HIGH INTENSITY ULTRASONIC PROCESSOR

High Tech Model
60-Watt

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The Ultrasonic Processor supplied with this instruction manual is constructed of the finest material and the workmanship meets the highest standards. It has been thoroughly tested and inspected before leaving the factory and when used in accordance with the procedures outlined in this manual, will provide you with many years of safe and dependable service.

Rev. 2003

WARRANTY

Your Ultrasonic Processor is warranted and backed by the manufacturer for a period of **three years** from the date of shipment against defects in material and workmanship under normal use as described in this instruction manual. During the warranty period, the manufacturer will, at its option, as the exclusive remedy, either repair or replace without charge for material and labor, the part(s) which prove to be defective, provided the unit is returned to us properly packed with all transportation charges prepaid.

Ultrasonic probes are guaranteed against defects for a period of one year from date of shipment. A defective probe will be replaced once without charge, if failure occurs within the warranty period. Wear resulting from cavitation erosion is a normal consequence of ultrasonic processing, and is not covered by this warranty.

This warranty is in lieu of any other warranties, either express, implied, or statutory. The manufacturer neither assumes nor authorizes any person to assume for it any other obligations or liability in connection with the sale of its products. The manufacturer hereby disclaims any warranty of either merchantability or fitness for a particular purpose. No person or company is authorized to change, modify, or amend the terms of this warranty in any manner or fashion whatsoever. Under no circumstances shall the manufacturer be liable to the purchaser or any other person for any incidental or consequential damages or loss of goodwill, production, or profit resulting from any malfunction or failure of its product.

This warranty does not apply to equipment that has been subject to unauthorized repair, misuse, abuse, negligence or accident. Equipment which, shows evidence of having been used in violation of operating instructions, or which has had the serial number altered or removed, will be ineligible for service under this warranty.

All probes are manufactured to exacting specifications and are tuned to vibrate at a specific frequency. Using an out-of-tune probe will cause damage to the equipment and may result in warranty nullification. The manufacturer assumes no responsibility for probes fabricated by another party or for consequential damages resulting from their usage.

The aforementioned provisions do not extend the original warranty period of any product that has either been repaired or replaced by the manufacturer.

IMPORTANT SAFEGUARDS

READ BEFORE INSTALLING OR USING THE EQUIPMENT

Your Ultrasonic Processor has been designed with safety in mind. However, no design can completely protect against improper usage, which may result in bodily injury and/or equipment damage. Please observe the following warnings at all times, read the operating instructions carefully before operating the equipment, and retain this instruction manual for future reference. If the Ultrasonic Processor is used in a manner contrary to that specified in this instruction manual, the protection features designed into the unit may be impaired.

When mounting the probe, always clamp the upper portion of the converter housing. Never clamp the probe.

Make sure the Ultrasonic Processor is properly grounded via a 3-prong outlet.

High voltage is present in the power supply. Do not remove the cover. Refer all servicing to qualified service personnel.

To avoid electric shock, disconnect the electrical power cord before removing the cover prior to servicing.

Never operate the power supply unless it is connected to the converter.

Never secure anything to the probe, except at the nodal point (point of no activity).

Never touch a vibrating probe.

It is recommended that a sound abating enclosure or ear protection be used when operating the Ultrasonic Processor.



WARNING or CAUTION
Where you see this alert symbol and WARNING or CAUTION heading, strictly follow the warning instructions to avoid personal injury or equipment failure.



SECTION 1 – INSTALLATION

INSPECTION

Prior to installing the Ultrasonic Processor, perform a visual inspection to detect any evidence of damage, which might have occurred during shipment. Before disposing of any packaging material, check it carefully for small items.

The Ultrasonic Processor was carefully packed and thoroughly inspected before leaving our factory. The carrier, upon acceptance of the shipment, assumed responsibility for its safe delivery. Claims for loss or damage sustained in transit must be submitted to the carrier.

If damage has occurred, contact your carrier within 48 hours of the delivery date. **DO NOT OPERATE DAMAGED EQUIPMENT.** Retain all packing materials for future shipment.

ELECTRICAL REQUIREMENTS

The Ultrasonic Processor requires a fused, single phase 3-terminal grounding type electrical outlet capable of supplying 50/ 60 Hz at 100 volts, 115 volts, 220 volts, or 240 volts, depending on the voltage option selected. For power requirements, check the label on the back of the unit.



WARNING

For your personal safety, do not, under any circumstances, defeat the grounding feature of the power cord by removing the grounding prong.



INSTALLING THE ULTRASONIC PROCESSOR

The Ultrasonic Processor should be installed in an area that is free from excessive dust, dirt, explosive and corrosive fumes, and extremes of temperature and humidity.

SECTION II – OPERATION

PRINCIPLES OF ULTRASONIC DISRUPTION

The ultrasonic power supply converts 50/60 Hz line voltage to high frequency electrical energy. This high frequency electrical energy is transmitted to the piezoelectric transducer within the converter, where it is changed to mechanical vibrations. The vibrations from the converter are intensified by the probe, creating pressure waves in the liquid. This action forms millions of microscopic bubbles (cavities) which expand during the negative pressure excursion, and implode violently during the positive excursion. This phenomenon, referred to as cavitation, creates millions of shock waves in the liquid, as well as elevated pressures and temperatures at the implosion sites. Although the cavitation collapse lasts but a few microseconds, and the amount of energy released by each individual bubble is minute, the cumulative effect causes extremely high levels of energy to be released into the liquid. The larger the probe tip, the larger the volume that can be processed but at a lesser intensity. For information regarding the processing capability of each probe, consult the tables below.

NOTE

The Ultrasonic Processor is available with four probes – a 2mm (5/64”), a 3mm (1/8”) microtip, a 6mm (1/4”) microtip, and 13mm (1/2”) probe. All probes are fabricated from titanium and are autoclavable.

The standard 3mm microtip can process between 250 microliters and 10 milliliters.

The 2mm microtip is optional and can process between 100 microliters and 5 milliliters.

The 6mm microtip is optional and can process between 10 and 25 milliliters.

FUNCTIONS OF KEYS, CONTROLS, INDICATORS, AND CONNECTORS

POWER SWITCH	Applies power to the instrument. Illuminates when power is on.
START BUTTON	When depressed, activates the ultrasonics. Also resets the TIMER and ENERGY CONTROLLER to zero.
STOP BUTTON	When depressed, de-activates the ultrasonics.
TEST BUTTON	When depressed, allows the power supply to be tuned. When released, automatically subtracts power consumed by the transducer and horn to ensure that the power displayed on the wattmeter is the net ultrasonic power delivered to the sample.
TUNE CONTROL	Optimizes power supply performance by tuning the power supply to the converter/probe assembly. To adjust, use only a non-magnetic, flat-blade screwdriver.
TIMER ON/OFF	Enables/disables the timer function.
TIMER	Sets the duration of ultrasonic application in minutes and seconds.
PULSER	Sets the duty cycle. In the OFF position, the ultrasonic is continuous. In the ON position the ultrasonics intermittent. The ON cycle and OFF cycle are independent of each other and can be adjusted from .1 second to 5 seconds. Intermittent operation inhibits heat build-up in the liquid and provides more efficient processing by allowing the material to settle back under the probe tip after each burst.
WATTMETER	Indicates the net amount of watts being delivered to the liquid.
ENERGY CONTROLLER	Controls and monitors the net amount of energy (joules) (watts-sec) delivered to the liquid. In the ON position the energy controller is energized. In the OFF position, the energy controller is de-energized.
OUTPUT CONTROL	Controls the amplitude of vibrations at the probe tip. CAUTION: when using a 2mm (5/64") probe, never exceed "60" on output control

REAR PANEL	
OUTPUT CONNECTOR	Connects to converter cable
FOOTSWITCH CONNECTOR	Connects to footswitch cable.
FUSE(S)	Protects against electrical overload.
ELECTRICAL CONNECTOR	Connect to electrical power cord.

PREPARATION FOR USE

CAUTION

If the Ultrasonic Processor has been left in a very cold environment for a prolonged period of time, do not operate until it has reached room temperature.

To safeguard the fuse against failure, always switch the power supply off before connecting or disconnecting the footswitch plug.

1. Ensure that the **POWER SWITCH**, **TIMER SWITCH**, **PULSER SWITCH** and **ENERGY CONTROLLER SWITCH** set to **OFF**.
2. Plug the electrical line cord into the electrical outlet.
3. If the probe is not already assembled, observe step 4.
4. If the converter / probe assembly is not already assembled; using the wrenches provided, screw securely the probe into the converter.

CAUTION

Never place a washer between the probe and the converter.
Never apply grease to the mating surfaces or threads of the converter, probe, replaceable tip or microtip.

5. Mount the converter / probe assembly in a laboratory stand, secure the clamp to the upper section of the converter housing only. Do not secure the clamp to any other portion of the converter / probe assembly.
6. Connect the converter cable to the power supply.

NOTE

Should it become necessary to remove a probe, use the wrenches supplied. If the probe has been attached to the converter for a long period of time it might be necessary to use a vise. Be sure the vise has soft jaws or other means to prevent scratching. Secure the wide diameter portion of the probe in the jaws of the vise. Never grip the converter in the vise. Using a wrench, twist the converter off the probe. A tap of a hammer may be applied to the end of the wrench. Never attempt to remove the probe by twisting the converter housing, as this may damage the electrical connections within the housing.

TUNING

Tune the power supply in accordance with the following procedures each time a new converter or probe is used.

1. Ensure that the probe is not immersed in the liquid and that it does not come in contact with anything.
2. Set OUTPUT CONTROL TO “100”. (to “60” with the 2mm (5/64”) probe)
3. Set TIMER ON / OFF SWITCH to OFF.
4. Set POWER SWITCH to ON, and rotate the TUNE CONTROL clockwise or counterclockwise until **minimum** (not maximum) reading (usually less than 30) is obtained on the POWER MONITOR. If minimum reading cannot be obtained, make certain that the probe is tight. A loose probe will usually generate a loud piercing sound.
5. Set OUTPUT CONTROL to “60”. (to “40” with the 2mm (5/64”) probe)
6. Set POWER SWITCH to OFF.

CAUTION

The power supply should be tuned after the probe has reached operating temperature. When working with low or high temperature liquids, immerse the probe in the liquid for a few minutes, withdraw the probe out of the liquid, and **then**, tune the power supply.

Ensure that the optional micro cup horn does not contain any liquid when tuning the power supply.

USING THE ULTRASONIC PROCESSOR

The speed control on an automobile, can, to a certain extent, be compared to an Ultrasonic Processor. The speed control is designed to maintain the vehicles rate of travel constant. As the terrain changes, so do the power requirements. The speed control senses these requirements, and automatically adjusts the amount of power delivered by the engine in order to compensate for these ever changing conditions. The greater the terrain rate of incline and greater the resistance to the movement of the vehicle, the greater the amount of power that will be delivered by the engine to overcome that resistance.

The Ultrasonic Processor is designed to deliver constant amplitude. As the resistance to the movement of the probe increases, additional power will be delivered by the power supply to ensure that the excursion at the probe tip remains constant. Using a more powerful power supply will not deliver more power into the liquid. Rather, it is the resistance to the movement of the probe that determines how much power will be delivered into the sample.

The AMPLITUDE control allows the ultrasonic vibrations at the probe tip to be set to any desired level. Although the degree of cavitation required to process the sample can readily be determined by visual observation, the amount of power required cannot be predetermined. A sensing network continuously monitors the output requirements, and automatically adjusts the power to maintain the amplitude at the preselected level. The greater the resistance to the movement of the probe due to higher viscosity, deeper immersion of the probe into the sample, larger probe diameter or higher pressure, the greater the amount of power that will be delivered to the probe. Setting the AMPLITUDE control fully clockwise will not cause the maximum power to be delivered to the sample. The maximum power that the Ultrasonic Processor is capable of delivering will only be delivered when the resistance to the movement of the probe is high enough to draw maximum wattage.

This phenomenon can be demonstrated as follows: depress the probe down against a piece of wood. The greater the down pressure, and consequent greater resistance to the movement of the probe, the greater the amount of power that will be delivered by the power supply.

CAUTION

- Do** not operate the power supply unless it is connected to the converter.
- H**igh voltage is present in the power supply – Do not operate with the cover off.
- N**ever allow liquid to spill into the converter.
- N**ever allow a microtip to vibrate in air for more than 20 seconds.
- W**hen using a 2mm (5/64”) probe, never exceed “60” on OUTPUT CONTROL.
- Do** not allow the vibrating probe to come in contact with anything but the sample.
- Do** not operate the converter in an explosive, humid, or caustic atmosphere.

1. Set TIMER, PULSER, and ENERGY CONTROLLER switches to OFF.
2. If a footswitch is to be used, plug the footswitch cable into the FOOTSWITCH CONNECTOR (rear panel). Make sure that the plug is inserted forcefully all the way in.
3. Set POWER SWITCH to ON. The switch will illuminate.
4. Ensure that the power supply is properly tuned.
5. Activate the ultrasonics by depressing the START button. If the footswitch is used, depress the footswitch.
6. Set OUTPUT CONTROL as required.
7. Depress the STOP button.
8. With the probe in air (not immersed in a liquid, or touching anything), depress the TEST button. This step automatically subtracts the power consumed by the probe and the transducer from the total power; to ensure that the power displayed on the wattmeter is the net ultrasonic power delivered to the sample.
9. Set TIMER as required. If the timer is not going to be used, set timer ON/OFF switch to OFF.
10. Set PULSER as required. If the pulser is not going to be used, set pulser ON/OFF switch to OFF.
11. Set ENERGY CONTROLLER as required. If the energy controller is not going to be used, set energy controller ON/OFF switch to OFF.
12. Activate the unit by depressing the START switch. If the footswitch is used, depress the footswitch.
13. Using OUTPUT CONTROL, increase or decrease intensity as required, then depress the TEST button.

IMPORTANT

To ensure repeatability and accuracy, always depress the TEST button after changing the OUTPUT CONTROL setting.

NOTE

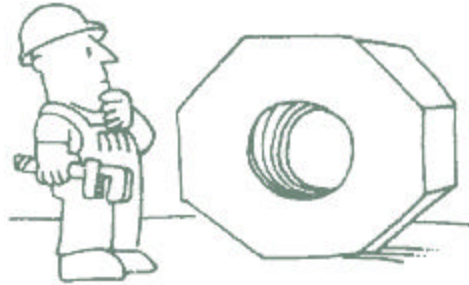
Whenever the pulser is used, the TIMER and ENERGY CONTROLLER will accumulate and register the time that has elapsed and the energy that has been delivered to the sample ONLY during the ON cycle. When working with a new application, or when time and energy settings are unknown, proceed as follows:

- a. Set TIMER to maximum (99 minutes / 59 seconds).
- b. Set ENERGY CONTROLLER to maximum (99999).
- c. Activate the unit by depressing the START switch. If the footswitch is used, depress the footswitch.
- d. When desired results are obtained, stop the ultrasonics and record the time displayed on the TIMER, and the amount of energy displayed on the ENERGY CONTROLLER.

The ultrasonics will automatically de-activate when the duration set on the TIMER has elapsed, or when the energy level set on the ENERGY CONTROLLER has been reached. The ultrasonics can also be de-activated at any time by depressing the STOP button.

As the volume or viscosity increases, so does the need for higher power output. A sensing network continuously monitors the output requirements, and automatically adjusts the power output in order to satisfy the needs of the application. The actual power delivered will only be that required by the application. The greater the resistant to the movement of the probe (resulting from higher viscosity or larger volume) the greater the reading on the WATTMETER.

SECTION III – SERVICE INFORMATION



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, or that it will stop working all together. The most probable causes for malfunction are listed below and should be investigated.

The unit was plugged into an electrical outlet that provides a different voltage from that required. See *Electrical Requirements*.

The probe is not secured properly.

If the probe has a replaceable tip, the tip is not secured properly, or the probe has been used with low surface tension liquids.

A fuse(s) has failed. If a fuse(s) has failed, proceed as follows:

1. Ensure that the power switch is set to OFF.
2. Open the fuse holder cover(s).
3. Replace the fuse(s).
4. Set the AMPLITUDE control to 50 and the power switch to ON. With the probe in air (out of sample), the wattmeter should read below 20 watts. If the reading exceeds 20 watts, set the power switch to OFF, and disconnect the probe from the converter.
5. Set the power switch back to ON. If the wattmeter reads below 20 watts, the probe has failed or is out of tune due to excessive erosion, and should be replaced. If the wattmeter reads above 20 watts, either the converter or power supply has failed and the complete Ultrasonic Processor should be returned for repair.

RETURN OF EQUIPMENT

It is suggested that an Ultrasonic Processor in need of repair be sent back to the factory.

In order to receive prompt service; always contact the factory before returning any instrument. Include date of purchase, model number and serial number. For instruments not covered by the warranty, a purchase order should be forwarded to avoid unnecessary delay. Care should be exercised to provide adequate packing to insure against possible damage in shipment. The Ultrasonic Processor should be sent to the “Service Department” with all transportation charges prepaid and return of shipment indicated.

Please obtain a *Return Authorization Number* prior to returning the instrument.

IMPORTANT

I CERTIFY THAT THE ULTRASONIC PROCESSOR AND / OR ACCESSORIES RETURNED FOR REPAIR ARE FREE OF ANY BIOHAZARDOUS OR RADIOACTIVE MATERIAL AND ARE SAFE FOR HANDLING.

DO NOT RETURN ANY EQUIPMENT UNLESS SUCH CERTIFICATION CAN BE MADE.

OPERATING SUGGESTIONS AND TECHNIQUES

DISRUPTING CELLS

The disruption of cells is an important stage in the isolation and preparation of intracellular products. From research levels through to production, many areas of biotechnology, particularly recombinant technology, necessitate the use of ultrasonics for cell disruption. Although some biological products are secreted from the cell or released during autolysis, many others require sonication to release intracellular material. Cell disruption focuses on obtaining the desired product from within the cell, and it is the cell wall that must be disrupted to allow cell contents extraction.

Single-cell organisms (micro-organisms) consist of a semipermeable, tough, rigid outer cell wall surrounding the protoplasmic membrane and cytoplasm. The cytoplasm is made up of nucleic acid, protein, carbohydrates, lipids, enzymes, inorganic ions, vitamins, pigments, inclusion bodies, and about 80% water. In order to isolate and extract any of these substances from inside the cell, it is necessary to break the cell wall and protoplasmic membrane. In some cases the cell may excrete the desired substance without assistance, but in most cases, the cells must be lysed and sonicated in order for these substances to be released. Breaking cell membranes and releasing the contents present significant challenges. The process must be fast and thorough to maximize the protein yield. Because the energy applied must be great enough to break the cell membranes or walls, yet gentle enough to avoid physically or chemically damaging cell contents, the Vibra-Cell with its variable intensity capability is ideally suited for this application.

The level of intensity that should be used is application dependent. For example high intensity might be recommended for the break up of cells, but should never be used when the release of intracellular components might be objectionable e.g. Organelle isolation.

The ability to control the amplitude at the probe tip is a prerequisite for process optimization. And because each application requires its own set of processing parameters, due to variation in volume and composition, the optimum amplitude can only be determined empirically. When processing a new sample, it is recommended that the amplitude be set first at 50% (30% with a microtip) and then increased or decreased as required.

Yeast, gram-positive bacteria, and to a lesser extent, gram-negative bacteria have considerably harder cell walls in comparison to animal cells, and require relatively high power for cell disruption.

Gram negative bacteria typically require 10 to 15 minutes of processing, while staphylococcus requires 20 to 30 minutes.

Microorganisms differ greatly in their sensitivity to ultrasonic disintegration. For example, the most readily disintegrated are the rod-like forms (bacilli), while the spherical organisms (cocci) are much more resistant. The group Mycobacteria, to which the tuberculosis organism belongs, is particularly difficult to disrupt. Generally, animal cells are more easily disintegrated than plant cells, and red blood cells are more readily disintegrated than muscle cells because they lack a protective cell wall.

Cellular disruption is the first step in RNA isolation and one of the most critical steps affecting yield and quality of the isolated RNA. Typically, cell disruption needs to be fast and thorough. Slow disruption, for example placing cells or tissue in guanidinium isothiocyanate (GITC) lysis solution for a long time prior to sonication, may result in RNA degradation by endogenous RNases released internally. This is especially a concern when working with tissues high in endogenous RNase such as spleen and pancreas.

Disrupting frozen tissue is more time consuming and cumbersome than processing fresh tissue, but freezing samples is sometimes necessary. Samples are usually frozen when, 1) they are collected over a period of time and thus, cannot be processed simultaneously; 2) there are many samples, 3) samples are collected in the field, or 4) mechanical processing of fresh samples is insufficient for thorough disruption. A mortar and pestle or bag and hammer are typically used when the starting material is frozen. RNA will remain intact in tissues for a day at 37°C, a week at 25°C a month at 4°C and indefinitely at subzero temperatures.

Ultrasonic processing will typically cause the temperature of the sample to increase especially with small volumes. Since high temperatures inhibit cavitation, the sample temperature should be kept as low as possible - preferably just above its freezing point. This can be accomplished by immersing the sample vessel in an ice-salt-water bath. Temperature elevation can also be minimized by using the pulser.

Increasing hydrostatic pressure (typically 15-60 psi) and viscosity can enhance cell disruption. For microorganisms, the addition of glass beads in the 0.5 to 1mm size range promotes cell disruption. Beads are almost a prerequisite when working with spores and yeast. A good ratio is one volume of beads to two volumes of liquid. Glass beads are available from Cataphote, Inc. P.O. Box 2369, Jackson, Mississippi 39225-2369 USA, phone (800) 221-2574 or (601) 939-4612, FAX (601) 932-5339, Jayco Inc. 675 Rahway Ave., Union NJ 07083 USA, phone (908) 688-3600, FAX (908) 688-6060 or Sigmund Lindner GmbH. P.O. Box 29. D-95483 Warmensteinach, Germany. Phone (49) 0 92 77 9 94 10, FAX (49) 0 92 77 9 94 99.

When processing difficult cells such as yeast, pretreatment with an enzyme is beneficial. Lysozyme, hyaluronidase, glycosidase, glucalase, lyticase, zymolase and lysostaphin digestion are among the enzymatic methods frequently used with yeast and Lysozyme with bacteria. Enzymatic treatment is usually followed by sonication in a GITC lysis buffer. Collagenase may be used with collagen, lysostaphin with staphylococcus, and trypsin hyaluronidase with liver and kidney.

If enzymes cannot be used, the following procedures should be considered: Freezing the sample at -70°C overnight, then thawing it in water immediately prior to ultrasonic processing.

Most animal tissues can be processed fresh (unfrozen). It is important to keep fresh tissue cold and to process it quickly (within 30 minutes) after dissection. When working with fresh tissue, the cells must be sonicated immediately at the time the GITC lysis solution is added. This can be done by dispensing the lysing solution in the tube, adding the tissue and immediately sonicating. Samples should never be left sitting in lysis solution, undisturbed. Large samples of hard tissues should be first treated in a blender or a mechanical homogenizer.

Animal tissues that have been frozen after collection should be disrupted by grinding in liquid nitrogen with a mortar and pestle. During this process, it is important that the equipment and tissue remain at cryogenic temperatures. The tissue should be dry and powdery after grinding. Grinding should be followed by thorough sonication in a GITC lysis buffer. Processing frozen tissue in this way is cumbersome and time consuming, but effective.

Cultured cells are normally easy to disrupt. Cells grown in suspension are collected by centrifugation, rinsed with PBS to remove culture medium, and then lysed by sonicating in a GITC lysis buffer. Placement of the vessel on ice while washing and lysing the cells will further protect the RNA from endogenous RNases released during the disruption process.

Soft, fresh plant tissue can often be disrupted by sonicating in a lysis buffer. Other plant tissues, like pine needles, need to be ground dry, without liquid nitrogen. Some hard, woody plant materials require freezing and grinding in liquid nitrogen prior to being ultrasonically processed. Plant cell suspension cultures and calluses can typically be sonicated in a lysis buffer within 2 minutes. The diversity of plants and plant tissue make it impossible to give a single recommendation for all. However, most plant tissues typically contain polysaccharides and polyphenols that can coprecipitate with RNA and inhibit downstream assays. Treating a plant tissue lysate with polyvinylpyrrolidone (PVP) will precipitate such problematic components from the lysate before the actual RNA isolation is carried out.

Whenever possible, the tissues should be diced very small to permit movement within the liquid. Tough tissues such as skin and muscle should be macerated first in a blender or the like for about 10 seconds, and confined to a small vessel during ultrasonic treatment. If sub-cellular particles are desired intact, the amplitude should be kept low, and the processing time increased.

Yeast can be extremely difficult to disrupt because their cell walls may form capsules or nearly indestructible spores. To process yeast, sonicate in a tube containing the sample, guanidinium-based lysis buffer and small glass beads (0.5 – 1 mm). Pretreatment with

zymolase, glucalase and / or lyticase to produce spheroplasts that are readily lysed may also be useful.

To disrupt filamentous fungi, scrape the mycelial mat into a cold mortar, add liquid nitrogen and grind to a fine powder with a pestle. The powder can then be thoroughly sonicated in lysis buffer to solubilize completely. As fungi may also be rich in polysaccharides, pretreatment with polyvinylpyrrolidone (PVP) may be beneficial.

Bacteria, like plants, are extremely diverse; therefore, it is difficult to make one recommendation for all bacteria. Ultrasonic processing will lyse most Gram positive and Gram negative bacteria, including mycobacteria. Although it is recommended that glass beads and lysis solution be used; it is possible to lyse some Gram negative bacteria by sonicating in lysis solution without beads. Bacteria cell walls can be digested with lysozyme to form spheroplasts. Gram positive bacteria usually require more rigorous digestion and longer processing time. The spheroplasts are then lysed with sonication in GITC lysis buffer.

Disruption of cells found in soil and sediments is accomplished one of two ways. One technique isolates the bacterial cells from the material prior to the RNA isolation procedure. This is accomplished by homogenization of wet soil in a mechanical blender followed by a slow speed centrifugation to remove fungal biomass and soil debris. The supernatant is centrifuged again at a higher speed to pellet the bacteria cells. Cells can then be lysed as described above for bacteria. Other techniques describe RNA isolation from the soil or sediment directly. For example, one method requires soil to be added to a diatomaceous earth and lysis buffer, and then sonicated. The sample is then centrifuged to remove solid debris.

Always immerse the probe deep enough below the surface of the sample to inhibit aerosoling or foaming, foaming substantially reduces cavitation. Processing at a lower power setting without foam is much more effective than processing at a higher power setting with foam. Decreasing the power, increasing processing time and lowering the temperature of the sample will usually prevent aerosoling and foaming. Do not use any antifoaming agents or surfactants.

During cavitation, free radicals are formed which, if they are allowed to accumulate, can greatly affect the biological integrity of the sample by reacting with proteins, polysaccharides, or nucleic acids. Although during short periods of processing their formation is not normally considered a problem; for longer durations, the addition of free radical scavengers such as, carbon dioxide, N₂O, cysteine, reduced glutathione, dithiothreitol or other SH compounds, might be beneficial. Saturating the sample with a protective atmosphere of helium or nitrogen gas, or dropping a small pellet of dry ice in the sample, will also inhibit free radical formation. Whereas it is true that gas is required for effective cellular disruption, it is not necessary that the vapor phase be oxygen or air since any gas except carbon dioxide will work just as well.

Various methods can be used to measure the efficiency of the disruption. For example, a visual count can be made using a microscope.

For greater accuracy, a protein assay could be used. This procedure is widely recognized as a good method for measuring cell disruption by taking into account the amount of protein released after disruption. The disrupted cells are then tested and checked against this number for percentage breakage.

There are several types of protein assays. One commonly used is the Folin Reaction (Lowry Assay) method, as it is comparatively simple and provides consistent results. This colorimetric method has a sensitivity to protein of around 8 µg / mL in the assay solution.

The assay turns blue in the presence of proteins due to the reaction of copper ions in the alkaline solution with protein and the reduction of phosphomolybdate- phosphotungstic acid in the Folin reagent by aromatic amino acids in the treated protein.

Fractional protein release, Rp, is calculated using the following equation and multiplying the result by 100:

$$R_p = \frac{C_f - C_b}{C_t - C_b}$$

Cf = Free protein

Ct = total protein

Cb = Background protein

This gives the actual disruption percentage, taking into account the background levels of protein before disruption.

Since the greatest concentration of energy is beneath the probe, it is imperative that the sample be kept as close to the tip as possible, liquids are easily processed because the free moving cells circulate repeatedly below the probe. Solid materials however have a tendency to be repelled by the ultrasonic, and should be processed in a vessel large enough to accommodate the probe, yet small enough to restrict sample movement. For small samples, conical shaped test tubes are recommended.

Allowing the probe to contact the vessel will decrease the power output, and cause minute grey glass particles to migrate into the sample. Although these glass particles will not adversely affect the chemical composition of the sample, they will form a thin grey layer on centrifuging. If the probe has to come in contact with a solid sample, use a standard 20mm (3/4") diameter stainless steel centrifuge tube cut to 70mm (3") length. Do not use a glass tube. Microtips must never allowed to come in contact with anything but the liquid, because the stress resulting at the point of contact with a hard surface will cause the microtip to fracture. Although larger probes will not fracture if they come in contact with a glass vessel, they may cause the vessel to fracture.

Before each application, place the tip in water or alcohol and energize the power supply for a few seconds to remove any residual substances. If concerned about contamination from previous use, clean the probe with a 20% Virkon solution and rinse with distilled water. For critical application, probes may be autoclaved.

To inhibit sample loss in test tube due to sticking, siliconize the test tube as follows: Wash and dry the test tube thoroughly, coat with silicone, then air dry. “Sigmacote” manufactured by Sigma Chemical Co., 3050 Spruce Street, St. Louis, Missouri 63103, USA, phone (314) 771-5765, is ideally suited for that purpose.

High viscosity and concentration are problematic. 2,000 cps and 15% concentration by weight are maximum limits. Because with ultrasonics the sound waves are propagated through the sample, if the sample is so thick that it will not pour or circulate easily, it is too thick for ultrasonic processing.

Use the Cup Horn for processing pathogenic, radioactive, and biohazardous materials in complete isolation without probe intrusion. Because plastic tubes have a tendency to absorb vibrations, it is preferable, whenever possible, to contain the sample in a stainless steel tubes or glass tubes when working with a cup horn. To expedite processing, add glass beads to the sample. If desired, crushed ice can also be added to the water inside the cup horn, in order to optimize cooling. Processing samples in a Cup Horn will usually take 4 times longer than processing with the direct probe intrusion method