BioSurface Technologies Corporation

Biofilm Annular Reactor (BAR) Operator's Manual

Models: BAR 1420 LS, BAR 1420 LJ

BAR 1420 LJ
Jacketed Laboratory Biofilm
Annular Reactor

421 Griffin Drive, Suite 2, Bozeman, MT, 59715, USA | biofilms.biz | (406) 585-2812

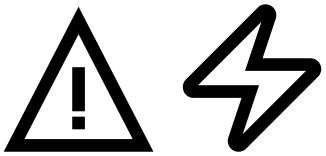
Contents

Operations Notice

Operation of this equipment in any fashion other than recommended by BioSurface Technologies Corporation will void the warranty. If damage or wear should occur while this equipment is being used in a manner not recommend by BioSurface Technologies Corporation, repair costs will be the responsibility of the user.

If you are unsure as to the proper installation and operation of this equipment, check the Operator's Manual or contact BioSurface Technologies Corporation at:

Phone: (406) 585-2812
Email: info@biofilms.biz



Motion Controller Connections Warning

Always power "OFF" the motor controller before making any electrical connections. Do not connect or disconnect electrical plug connection between the motor controller and voltage transformer while the controller unit is "ON".

Model 1420 Biofilm Annular Reactor (BAR) and Water Quality Monitor

1.0 General Description

The BAR continuously measures the accumulation of deposits on a surface in contact with flowing liquids by providing readily available sampling surfaces.

Accumulation of any type of film, whether microbial, organic, or inorganic can be measured. See Appendix A for a listing of Reference Literature.

The BAR is specifically designed to monitor any type of microbial accumulation. The basic BAR System is divided into two general types, the standard laboratory unit and the jacketed laboratory unit that allows circulation of heating or cooling fluid in an outer jacket.

2.0 Monitor Construction

The BAR consists of two concentric cylinders: a stationary outer cylinder and a rotating inner cylinder. Process fluid moves in the region between the cylinders with biofilms accumulating on the internal surfaces. Rotational speed (30 to 500 RPM) is controlled by a variable speed DC motor (100 to 240 VAC). Twenty removable slides are flush mounted on the internal, rotating cylinder. Slides (coupons) for obtaining deposit samples can be constructed from most relevant materials. See Section 9.0 and Appendix B for Parts and material lists.

Research and process monitoring benefits of the BAR include the following:

1. Kinetic analysis of biofilm rate processes, including nutrient uptake, growth rate, and biofilm removal.
2. Removable growth surfaces permit intermittent monitoring and measuring of cell mass, number, and biofilm composition.
3. Minimum biomass growth in the bulk fluid at short fluid retention times.
4. Controlled fluid shear, independent of bulk residence time.
5. Compact construction; minimum space requirements.
6. Simple, reliable operation with low maintenance.
7. Ideal for biocide studies.
8. The Model 1420 Biofilm Annular Reactors can be sealed to allow for the study of pathogenic biofilms.

Figure 1 – BAR 1420LS Standard Laboratory Biofilm Annular Reactor

Figure 2 - BAR 1420LJ Jacketed Laboratory Biofilm Annular Reactor

3.0 Applications

The primary function of the BioSurface Technologies BAR is to provide a platform for biofilm evaluation in water and process fluids. Although its application may vary from user-to-user, the Biofilm Annular Reactor is ideally suited to mimic water distribution systems or industrial piping systems because of its ability to simulate hydraulic residence times and hydraulic shear stresses. Some specific applications are presented in the following sections.

3.1 Drinking Water Applications

As result of the 1986 Amendments to the US Safe Drinking Water Act (SDWA), many utilities are faced with the difficult task of balancing disinfection by-products and microbial disinfection. Analytical techniques are currently available that enable utilities to estimate the formation potential of disinfection by-products, but not the long-term regrowth potential of bacteria in the distribution system. The BAR can be used to evaluate the long-term microbial regrowth potential in the distribution system. Potential drinking water applications of the BAR include:

- Evaluating the disinfection efficacy between free chlorine and alternate disinfectants against attached versus suspended microbial activity in distribution systems.
- Evaluating the long-term disinfection efficacy of reduced disinfectant residuals in distribution systems.
- Evaluating the efficacy of potential disinfectant booster stations in distribution systems.
- Evaluating the distribution regrowth potential of treatment plant modifications. This evaluation will require the use of pilot plant facilities.
- Evaluating the long-term impacts of various corrosion inhibitors against pipeline corrosion and microbial regrowth. Most corrosion inhibitors contain phosphorus which is a valuable nutrient for microbial growth.

Typical flow schematics using the BioSurface Technologies BAR are available on request.

3.2 Industrial Applications

The uses of the BAR will vary significantly for various industrial applications. Some potential applications of the Biofilm Annular Reactor in industrial facilities include:

- Evaluating biocide efficacy in cooling towers and heat exchangers.
- Conducting biostability studies for various process waters.
- Conducting corrosion control studies for various process waters.
- Evaluating control of biofilm growth in ultrapure water distribution systems.

Typical experimental schematics are shown in Figures 9, 10, and 11.

4.0 Protocols for Typical Installations

It is important that the bar be operated to simulate your process system correctly. To assist users establishing operational criteria that will simulate hydraulic and chemical conditions in the distribution system, a typical testing protocol is presented below:

1. Establish typical operating criteria of the distribution system (disinfectant dose, corrosion inhibitor dose, finished water pH, typical water temperature, typical pipe size, typical pipe velocity, typical Hazen-Williams coefficient).
2. Determine the operating speed of the BAR that will simulate the shear stress present in the distribution system. The shear stress is directly related to pipe size, flow velocity, and the hydraulic gradient of the distribution system. The rotational speed of the BAR can be determined by the operating tables in Appendix D. Rotational speed can be determined using the RPM meter or for speeds of less than about 20 RPM by counting the revolutions (observe the white nylon spot on the top of the reactor as seen through the top plate).
3. Set-up the BAR and other ancillary equipment/facilities (tubing, influent water source, chemical feed pumps, chemical storage containers, etc.) as required for the desired evaluations.
4. Determine the hydraulic retention time in the regrowth monitor. An initial minimum retention time of 24 hours is typically required to establish microbial surface growth, after which the retention time is decreased to less than 2 hours (depends on volumetric flow through section of distribution system being modeled, media use concerns, etc.).
5. Disinfect or sterilize all tubing and monitor in accordance with Section 5.5 or 5.6, respectively.
6. Determine chemical feed rates and chemical solution concentrations. Chemical solution concentrations should be prepared to allow feed rates within the operational ranges of your pumps and consistent minimal media preparation (usually not less than 0.25 ml/min or higher than 0.5 ml/min).
7. Calibrate all chemical feed pumps and influent water source to the calculated flow rates.
8. Initiate operation of the BAR and ancillary equipment/facilities (inoculate with desired bacterial population if sterile dilution water and medium are added).
9. Check chemical feed rates and chemical feed concentrations daily. The frequency of calibrating chemical feed solutions and feed rates can be reduced once the ancillary equipment have demonstrated consistency. Disinfectant solutions should be stirred frequently (daily) to dissolve any vaporized or separated disinfectant within the disinfectant solution container.

10. Collect effluent and biofilm samples on a routine schedule. Effluent samples can be collected from the dis- charge of the BAR or removed through an aseptic port on the top of the monitor (recommended). Biofilm samples can be collected by removing the coupons from the regrowth monitor in accordance with Section 10 of this manual.
11. All effluent and biofilm samples should be dated and recorded on the appropriate data forms.

The BioSurface Technologies Corporation Biofilm Annular Reactor system has been designed for a maximum influent flow rate of approximately 50 ml/min. Higher influent rates are possible with minor modifications. Contact BioSurface Technologies Corp for details.

5.0 Assembly and Care

The Model 1420 BAR system is shown on the cover of this document. A detailed parts list for the Model 1420 BAR is provided on page B-1. The assembly of the system includes the following:

5.1 Assembly of the BAR

The BAR has been partially disassembled for shipping to prevent damage. The following steps will be followed to assemble the BAR:

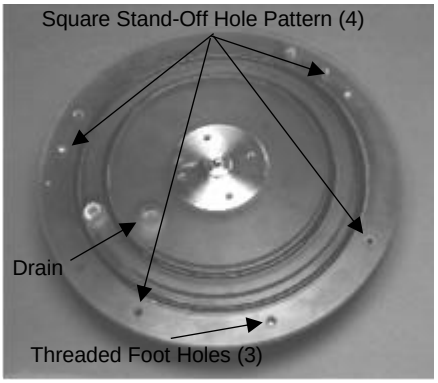


Figure 1 - Base Plate Assembly

1. Place the Base Plate Aluminum Support (PN# BST1122A) on a sturdy bench surface. Place the Base Plate (PN# BST 1119A) on the aluminum support and align the discharge ports on the base plate with the corresponding holes on the aluminum support. Make sure the stand-off holes in the base plate aligns with the threaded holes in the aluminum support plate (square hole pattern; Fig. 3). Thread the plastic knurled head screws (feet) into the aluminum support from the bottom into the three threaded holes closest to the plate edge (triangular hole pattern; non-stand-off holes).
2. Place the knurled brass nuts on the bottom of the thread- ed stand-offs locating them approximately 1 inch from the end. Place the stand-offs through the bottom plate holes (through holes; no threads in the polycarbonate base plate) and thread them into the aluminum base plate for approximately 3 turns (until they are even with the bottom of the aluminum stiffener plate). Lightly tighten the brass nuts back down against the base plate.
3. Place the ball bearing in the milled cup on the bottom plate ball tower (Fig. 5). Place the inner cylinder on the ball bearing positioned on the ball tower. Align the

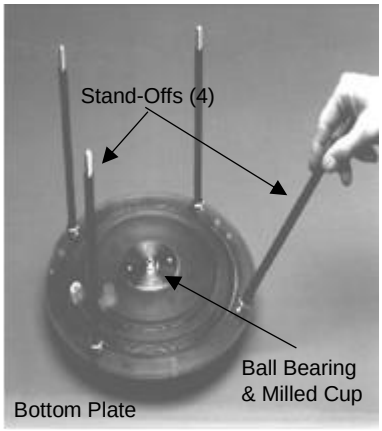


Figure 4 – Stand-Off and Base Plate Assembly

bottom milled cup on the inner cylinder assembly with the milled cup/ball-bearing on the base plate.

4. Place the outer cylinder (glass) on the base plate/ O-ring. Visually align the outer cylinder so it is essentially equidistant from the base plate machined surface (inside of the O-ring groove) and over the O-ring. This location will minimize shear force variations by keeping the outer cylinder surface equidistant from the rotating inner surface. Place the glass water jacket on the base plate, aligned on the outer O-ring equidistant from the glass outer cylinder (BAR 1420 LJ only).
5. Carefully place the Top Plate (BST-1118A; see Figure 7) on the inner cylinder shaft and align with the Threaded Steel Rods. Be careful to avoid catching the inner cylinder shaft on the shaft lip seals when installing the Top Plate. The Top Plate may require rotation to align the holes with the threaded rod.
6. Place aluminum ring (BST-702 Plate Stiffener) on the top plate, making sure stand-off supports align with holes in the aluminum ring. The beveled hole-plug opening faces up to allow hole-plug removal clearance. This ring will evenly distribute the pressure around the glass cylinder and provide a tight seal up to approximately 10 psig over-pressure. If knurled nuts do not contact stand-offs for at least 3 complete turns, repeat step (b) to further thread or unthread the stand-offs in the bottom aluminum plate (Figure 7).

5.2 Mounting the Motor/Drive Unit

The motor/drive unit mounts to the top of the BAR (Figure 8). Place the metal shaft coupler onto the inner cylinder shaft and align the shaft flat spot with the set screw in the coupler. Tighten the set screw onto the shaft using the hex wrench provided. This requires tightening and loosening the set screw until the coupler slide slides onto the shaft. Align the set screw with the flat spot on the shaft. There should be minimal movement of the set screw across the flat section of the shaft. Place the neoprene sleave onto the shaft coupler. Place the motor onto the motor mount plate while aligning the neoprene coupling collar to the metal shaft coupler on the top of the reactor and bottom of motor drive unit. Thread the motor mounting thumb screws into the plate to securely attach the motor to the reactor.

5.3 Inner Cylinder and Motor Alignment

The inner cylinder alignment is necessary each time the unit is assembled. Improper alignment will result in excessive wear on the top seals and the motor shaft.

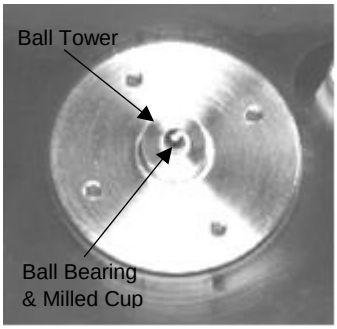


Figure 5 – Ball Tower and Ball Bearing

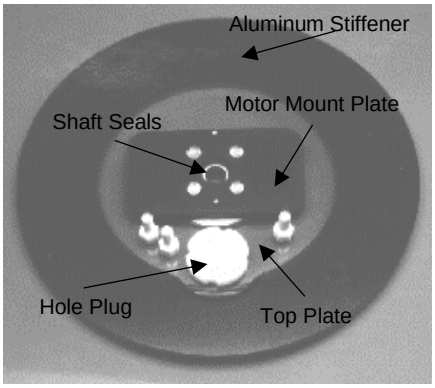


Figure 6 – Top Plate Assembly

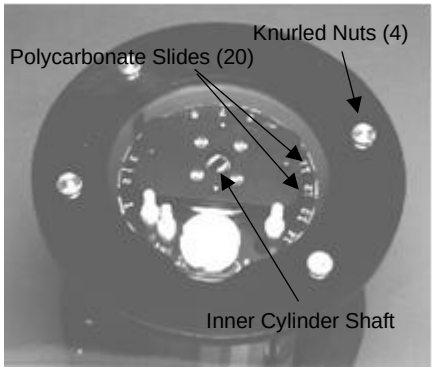


Figure 7 – Assembled Reactor, Top View

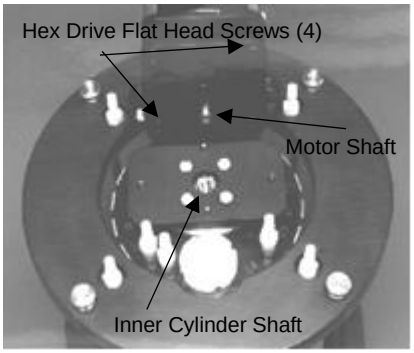


Figure 8 – Shaft Alignment

To align the inner cylinder, loosen (but do not remove) the top brass knurled nuts so they no longer engage the top anodized aluminum ring (do this adjustment when the unit is empty of fluid). Turn the power on and adjust the rotation to ~100 RPM. While the unit is rotating, adjust the upper plate to minimize noise and vibration. Visually note the eccentricity of the inner cylinder wobble and adjust the position of the upper plate to minimize the wobble (may require slightly adjusting outer cylinder placement to provide a concentric assembly). After minimizing the wobble, lightly tighten the brass knurled nuts (tighten opposite sides to create even seal around rim).

5.4 Fluid Influent and Reactor Venting

Fluid influent is through 1 or more of the hose barb fittings on the top plate of the unit. A bacterial vent filter should be attached to the effluent tube leg that is open to the atmosphere to prevent contamination. The Bacterial Air Vent may become plugged with water vapor after autoclaving or if water is allowed to enter the filter housing.

Plugging of the air vent may cause siphoning of the reactor contents. BioSurface Technologies Corp. recommends verifying easy air flow through the vent prior to installation and operation of the air vent on the reactor effluent line after autoclaving (use compressed air to blow back through the vent to ensure unimpeded gas flow).

The bacterial vent is used only if containment of pathogens within the reactor, or defined bacterial cultures are required within the reactor. If the bacterial population within the reactor is undefined and considered non-pathogenic, the air vent may not be required.

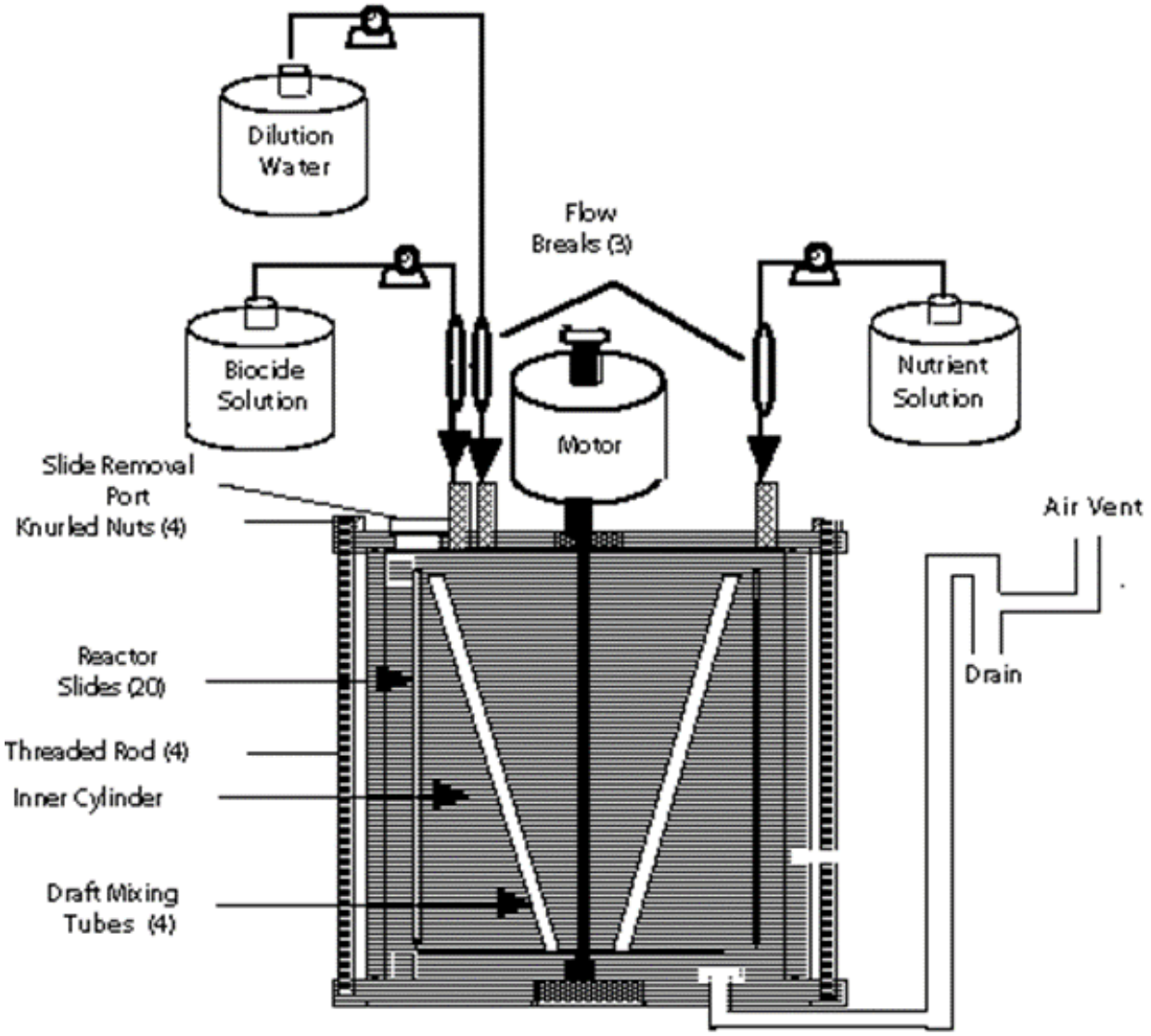


Figure 9 – Continuous Flow (Once Through)
Laboratory BAR System Schematic

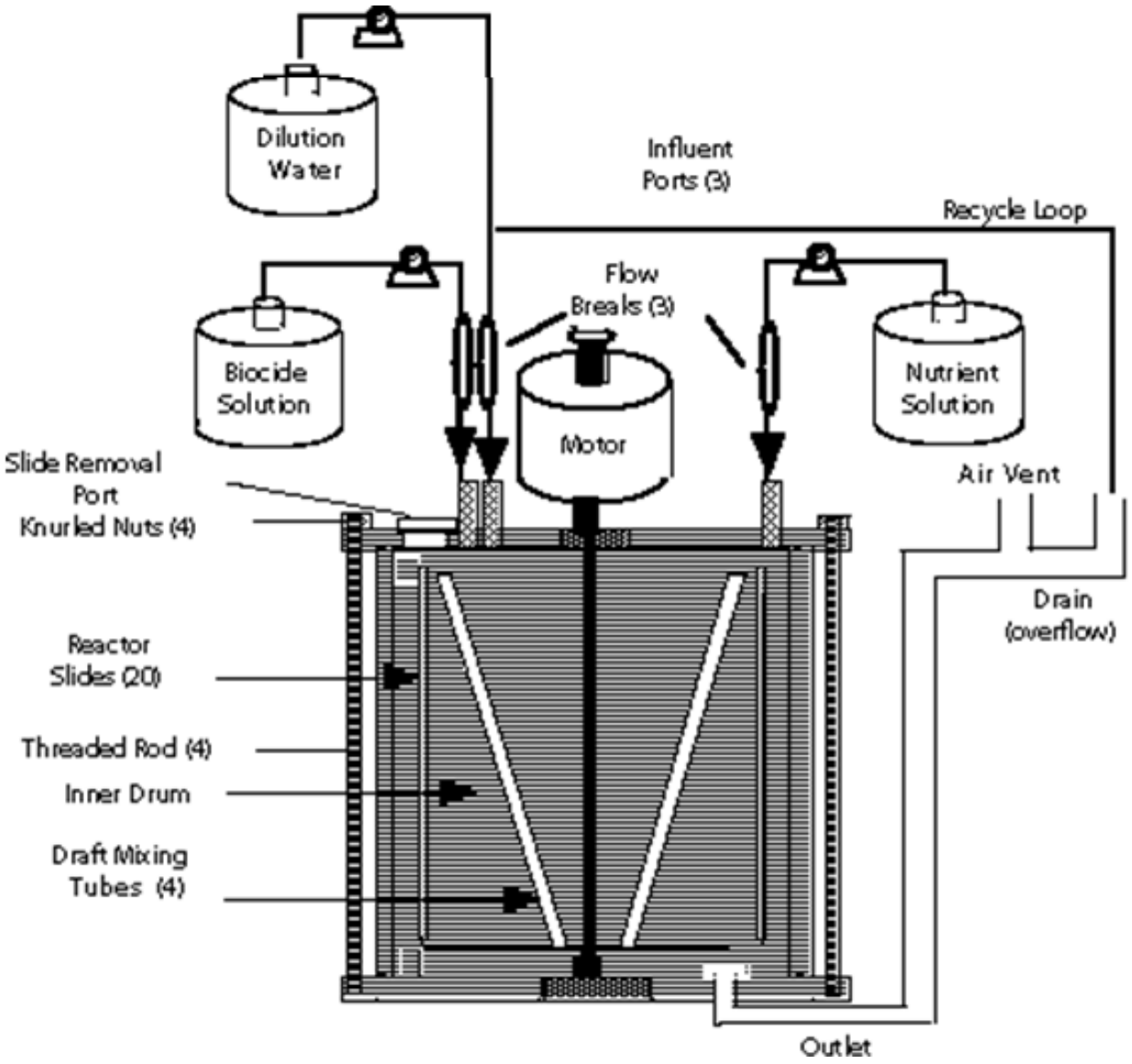


Figure 10 – Continuous Recycle Laboratory
BAR System Schematic

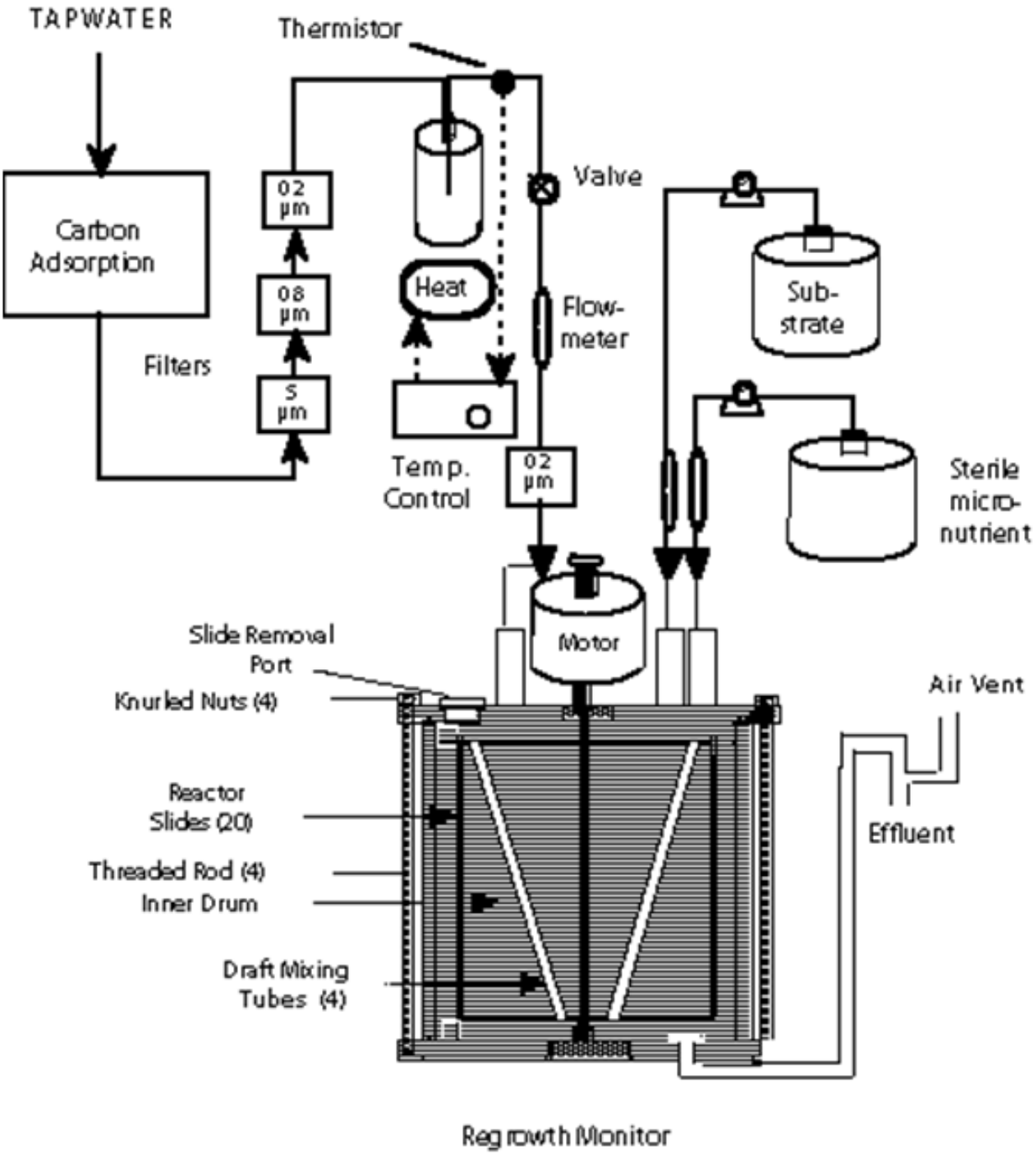


Figure 11 – Experimental Example
Laboratory BAR System Schematic

5.5 Removal of Slides

The Model 1420 BAR will accommodate 20 removable slides. See Figure 12. These slides can be manufactured from plastics (polycarbonate supplied), or any machinable metal, such as mild steel or stainless steel (full list of materials available can be found on www.biofilms.biz). On occasion the slides will become "sticky" and difficult to remove (test before starting your experiment). If this occurs, lightly sand the beveled edge with emery paper. The slides are inserted and removed with the provided hooked tool inserted in the hole in the top of the slide. Use the motor potentiometer to slowly turn the inner cylinder to properly align the inner cylinder slot with the top plate hole for slide removal. (the slide removal tool can also be sterilized and inserted to rotate the inner cylinder to facilitate slide removal).

Figure 12 – Inner Cylinder with 20 Slide Coupons

5.6 Motor Controller

The motor controller unit contains the power on/off switch, the RPM indicator, the motor control board, and the motor speed potentiometer. After connecting the motor control unit to the voltage transformer, plug in the power cord and turn the unit on. The rotational speed may be adjusted using the speed potentiometer as monitored by the RPM indicator. Your motor control unit should not be opened under normal operating conditions. If the motor does not operate (check when the motor is not mounted on the Regrowth Monitor), first check your power source. If power to the unit is verified and the unit does not operate, check the fuses on the side of the motor control unit. (disconnect from power before removing fuses). See the Trouble Shooting Section for fuse replacement.

Figure 13 – 1420 Motor/Control Unit

5.7 Plumbing the BAR

The Laboratory version of the BAR can be used in either once- through (see Figure 9) or continuous recycle systems (see Figure 10). In both cases water and other additives, such as nutrients, biocides, pH control chemicals, etc. enter from the top and effluent leaves through the bottom. A flexible Tygon™ or similar tubing loops up from the bottom port and is open to the atmosphere, The discharge line Ts off this line level with the desired fluid level in the Regrowth Monitor. With high nutrient levels, this side port tends to become constricted due to biofouling. It should be squeezed periodically during a run to loosen and remove the fouling materials. The fluid in the Regrowth Monitor (approx. 1000 ml) should be maintained to cover the inner cylinder. A flow break has been provided to minimize biofilm growth up the nutrient supply line. The flow break should be located just above the reactor and autoclaved in place with the unit (remember to cover all open tubing lines with autoclave paper or tin foil).

Tygon™ tubing fits onto the hose barb fittings on the top and bottom plates of the monitor. The effluent line (bottom hose barb) requires a “Tee” fitting even with the top of the reactor to allow adjustment of the water level inside the reactor. One leg of the Tee is left open to the atmosphere to prevent siphoning of the reactor fluid through the effluent line (vented using the Bacterial Vent Filter provided when operating with potential pathogens or defined cultures). A small length of tubing may be required on this leg above the Tee to allow sufficient head to

develop to force the effluent out the drain line. The remaining leg of the “T” requires a length of tubing to reach a waste container or drain. See Figures 9-11. Fluid influent is through 1 or more of the hose barb fittings on the top plate of the unit.

Plugging of the air vent may cause siphoning of the reactor contents. BioSurface Technologies Corporation recommends verifying easy air flow through the vent prior to installation and operation of the air vent on the reactor effluent line after autoclaving. The bacterial vent is used only if containment of pathogens within the reactor, or defined bacterial cultures are required within the reactor. If the bacterial population within the reactor is undefined and considered non-pathogenic, the air vent is not required.

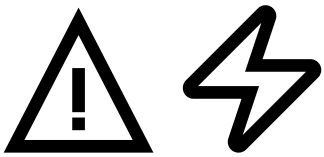
5.8 Tubing Support

A black anodized rod female threaded at one end to allow attachment to the top of one of the stand-offs. This post can be used like a chemistry support stand to act as a tubing support.

5.9 Autoclaving Recommendations

The Model 1420 BAR is made of polycarbonate, stainless steel, glass, and Kevlar/nylon. The regrowth monitors are made to be fully autoclavable. However, extensive autoclaving may decrease monitor longevity due to increased stress on components. In the Model 1420, it may be necessary to autoclave the reactor prior to any experimentation. To do this, assemble the reactor as described in the Assembly Section, (minus motor control unit). During autoclaving, do not overtighten knurled nuts holding aluminum ring and top plate in position. Finger-tighten knurled nuts until snug, and then loosen 3/4 turn just prior to autoclaving. Loosen the knurled nuts on the base of the standoff rods and retighten after autoclaving. Leave at least 1 influent or effluent line open to the atmosphere to allow free steam access (cover the end of the tube or port with autoclave paper and tape in place with autoclave tape).

Place a small amount of water in the reactor and attach any necessary tubing to the reactor. Place the assembled reactor upright in the autoclave and autoclave for 40 minutes at 121oC (15 psi). After autoclaving, allow adequate time for the reactor to cool before connecting the motor drive assembly. Loosen all brass finger nuts to minimize stress and possible damage to the reactor when placing into the autoclave. Re-tighten after removal from the autoclave and as the unit cools.



The Motor Drive/Control Unit should never be autoclaved or placed in an environment where it may become wet as it will create a hazardous electrical condition due to the exposed electronics.

5.10 Disinfecting the Regrowth Monitor

The Regrowth Monitor can be disinfected rather than steam sterilized. After the monitor has been assembled, slides cleaned/replaced, and fouled tubing replaced, the entire system should be disinfected. To do this, prepare a 2-liter carboy with a 70% solution of ethanol.

CAUTION: This solution is extremely flammable. Connect the carboy to the system using the recycle set up shown in Figure 7. Allow the solution to cycle through the entire monitor system for 2 hours, with all circulation pumps and the reactor motor on. This is a good time to check for any leaks in tubing, etc. After this time, drain all ethanol solution from the system and allow about 3 system volumes (approximately 3 liters) clean sterile water to flush the system (do not recycle, fill completely and then drain, repeat procedure three times). After the flush, the system is ready for use.

5.11 Cleaning Recommendations Between Experiments

The BAR is made for quick and easy cleanup between experiments. To clean, drain all fluid from the unit and remove all connected tubing. Remove the monitor vessel from the motor drive assembly to prevent water damage to the electronics. Disassemble the BAR vessel by removing the 4 knurled brass nuts, gently sliding the top aluminum and polycarbonate plate off the steel support rods and inner cylinder shaft. Then remove the water jacket, (BAR 1420 LJ), outer glass cylinder and the inner cylinder. Place all components in a solution containing a laboratory-strength cleaning solution. Using a nylon brush, scrub the inside of the outer cylinder, the top and bottom plates (be careful not to dislodge and lose the ball bearing) as well as the outside of the inner cylinder and the draft tubes.

After scrubbing, discard solution and add fresh solution to the tub or sink. Allow to soak for approximately 2-3 hours, making sure that all components are submersed. If rigorous disinfection is needed, a 5% glutaraldehyde soak is recommended in lieu of the soap for the same amount of time (be certain to use proper ventilation). New slides should be used for each new experiment. Please contact BioSurface Technologies for further information. After the BAR has been thoroughly cleaned and rinsed, allow components to air dry, then reassemble as described in Assembly Section. Replace any fouled tubing leading into or away from the monitor vessel as well before reassembly.

5.12 Maintenance

Make certain that the BAR race-bearings (top plate bearing housing) are not exposed to water (except during cleaning or autoclaving). These bearings will occasionally need replacing based on use and high humidity. The shaft seals located on the underside of the top plate bearing housing will also require periodic replacement. See Troubleshooting.

6.0 Troubleshooting

1. The regrowth monitor stops when power is on:
    - Turn power off and check the two fuses located on the side of the motor/control chassis. Two extra fuses are provided with each unit in the spare parts. Please contact BioSurface Technologies for further instructions (tech@biofilms.biz). Fuses should not blow under normal operating conditions.
2. The unit leaks at O-ring seals at the top or bottom of the glass cylinder and/or water jacket (BAR 1420 LJ):
    - Tighten the brass thumbscrews on the top flange of the regrowth monitor. Do not ap- ply excess force on the brass thumb screws that may cause deflection and damage of the top plate. Tighten opposing side thumbscrews. Pliers may be used to assist. Do not tight so much as to deflect the top plate.

- Disassemble the unit and check the seating of the O-ring seal. Re-seat or replace the O-ring seal if necessary.
- Leaks may occur in one of two places. First, around the upper and lower O-rings on the top and bottom plates as they seal against the glass Outer Cylinder. This seal should be capable of maintaining approximately 10 psi. If leaks persist after tightening uniformly, check for chips or imperfections on the glass outer cylinder. Replace glass and O-rings are required.
- The Model 1420 LJ Jacketed BAR. If leakage occurs into the jacket annulus from the inner reactor, or out of the jacket while the inner reactor is sealed, a thin spacer gasket can be used to generate a seal to the problem O-ring seal. The jacketed BAR uses concentric glass cylinders. These cylinders are precision ground to length to allow a compression seal on both cylinder rims simultaneously. Occasionally the tolerance differences between these two cylinders require placement of a thin flat spacer gasket under one or the other of the O-ring seals. This thin spacer gasket will provide the necessary additional seal. The thin seal is placed under either the glass water jacket bottom O-ring, or the glass cylinder bottom O-ring, depending on where leakage is occurring. All reactors are tested prior to leaving our facility and are provided with a working seal. When replacing either the outer glass cylinder or Water Jacket Glass Cylinder, this thin spacer gasket may be required.

3. The Inner Cylinder does not appear to fit properly or is tight:
    - The reactor should not require excessive tightening of the knurled nuts to seal the outer cylinder with the top and bottom plate O-rings. If the reactor will not seal without excessive tightening of the knurled nuts, inspect the O-ring seals in the top and bottom plates and the glass cylinder contact surfaces. Make sure the O-ring and glass end contact surfaces are clean and are free of damage or obstructions. See section 2 above concerning BAR 1420 LJ.
4. The inner cylinder does not rotate freely or smoothly.
    - The inner cylinder should rotate freely on the ball bearing support (before mounting the top plate). If the cylinder does not rotate freely, remove the inner cylinder and its supporting ball bearing. Check the ball bearing for pitting or corrosion, replace if necessary. Check the milled bearing bushing (yellowish polymer in the bottom bearing plate support and bottom of the inner cylinder shaft) for excessive wear, pitting, or deformation. Replace if excessive wear is evident. If the slides contact the top plate after tightening the top knurled nuts, contact BioSurface Technologies for instructions.
5. The motor vibrates or catches as the inner cylinder rotates.
    - The alignment of the motor shaft on the inner cylinder shaft is critical for proper operations. If the inner cylinder does not rotate freely, or if the motor assembly wants to vibrate or "walk" on the top bearing housing platform, the motor assembly alignment is not optimal. No noticeable vibration or rotational fluctuations should occur during normal operations. If you continue to have problems with the rotational vibrations or motor vibrations, contact BioSurface Technologies Corporation.
6. The inner cylinder shaft does not rotate freely.

- The inner cylinder shaft should rotate without catching when the motor assembly is not mounted on the reactor. If the inner cylinder does not rotate freely, and the alignment procedures have been followed, check the ball bearing for wear that would impede rotation. If these components are okay, the bearing and seals may need replacement. Contact BioSurface Technologies Corporation.

If you continue to have problems, please contact:

BioSurface Technologies Corporation
Phone: (406) 585-2812
E-mail: Tech@biofilms.biz

7.0 Slide Materials Use

Different types of slides are available for the BAR 1420. Go to our website (biofilms.biz) for material options.

Cast iron slides that closely match cast water pipe are available for the BAR. This material is extremely brittle and requires a slide thickness about 0.25 inches to prevent handling breakage. These slides can be used on any Model LS or LJ reactors but require a modification to the inner cylinder to accept the thicker slide. Standard (thin) slides can also be used on the modified monitors but require a spacer slide behind the thin slide to reduce the volume of the reactor and prevent excessive growth of biofilms behind the thin slide.

7.1 Installation of Slides

The beveled edges of the standard slides drop into the beveled slots on the reactor inner cylinder.

The cast iron slides have been machined to provide a “T” profile. These slides drop into the formed notches in the inner cylinder. The slide fit has been purposely provided slightly undersized to allow easy installation of the slides into the slots, and to allow for corrosion product buildup. The hole in one end of the slide must always be located at the top of the reactor.

7.2 Slide Removal

Standard slides are removed by using a hook or wire (flamed to sterilize) using the procedure defined in Section 11.1.

Removal of the cast iron slides after immersion in water may require a slight tap with a hammer and punch. The slides will weld into the slots due to corrosion product build-up. A rubber gasket has been provided at the bottom of the inner cylinder to allow slight slide movement downward to “break” the slides loose.

After breaking the corrosion weld of the slide in the slot, the slides can be removed using the hooked tool provided by inserting the barbed end of the tool into the hole in the top of the slide and pulling the slides straight up.

7.3 Inner Cylinder Alignment

WARNING! Do not rotate the regrowth monitor without a full complement of slides at high speed. The imbalanced weight of the inner cylinder will cause excessive wear

on the seals and bushing and may cause catastrophic damage to the regrowth monitor (primarily applicable to cast iron slides).

8.0 Motor/Controller Operation

The Model 1420 has a single, sealed motor and control box, and it is not advised to disassemble in a laboratory environment. Contact BioSurface Technologies Corporation for component repair or replacement of the motor and motor controller.

9.0 RPM Computer/Indicator

The BioSurface Technologies Corp. BAR is provided with a built-in RPM indicator. The display read-out represents the rotations per minute (RPM) of the polycarbonate inner cylinder. The displayed RPM may appear to change periodically due to computational cutoffs programmed inside the motor controller. The actual RPM of the device will remain steady and will be an average over time of what is displayed on the RPM read-out.

Some precise numbers may be unattainable on the display due to programming of the motor controller, which is unavoidable. A tolerance of 1 or 2 RPM is normal and expected.

10.0 Analysis Techniques

10.1 Removal of Coupons

Equipment needed:

- spray bottle containing disinfectant (70% EtOH is recommended but 1% bleach can be used but is extremely corrosive, and should be wiped clean with a damp cloth after sample port has been closed)

- sterile cell scraper

- 10 ml sterile phosphate-buffered saline (PBS or water— refrigerate until use)

- sterile 100 ml beaker

- disposable rubber gloves

- one ml pipet

1. Turn off drive motor so inner drum in no longer spinning.

2. Fog the sampling port with disinfectant.

3. After approximately 20 seconds, quickly open the sampling port, taking care to leave open only a minimal amount of time.

4. Quickly remove the desired coupon using the metal hook provided to slide the coupons out of the reactor. If inner drum is not in proper position to remove desired coupon, rotate inner drum slowly by turning shaft, noting engraved numbers on the inner drum. Note the exposed side of the coupon and coupon number.

5. Set the coupon on a flat surface, with one end propped up, exposed side up, then close sampling port.

6. Put on rubber gloves, wash in disinfectant, allow to dry for 20 seconds.

7. Open sterile beaker and pour 20 ml sterile PBS (or H2O) diluent into it (sample bottles containing 100 ml in a 125 ml Nalgene bottle work well. One bottle per sample).

8. Carefully lift coupon and scrape using sterile cell scraper into beaker. Rinse scraper in diluent. Repeat scraping and rinsing three times.

9. Using a five (5) ml pipet, rinse scraped surface using diluent.

10. Decant the fluid containing scrapings back into the original PBS (total volume = 100 ml) bottle using diluent for rinse. Take care to label vial with date, coupon position, etc.

11. Discard cell scraper but leave rubber gloves on if any further coupons are to be removed.

12. Repeat steps 2-11 for each coupon to be removed.

13. Turn on drive motor if experiment is not complete.

14. Transport all PBS/water bottles containing scraped samples to laboratory for immediate analysis. If transportation time is significant (>2 hours), portable refrigeration or storage on ice may be necessary during transport.

10.2 Scraping, Homogenizing, and Sampling Protocol

1. Decant the fluid from the previous step (9.1 Step 14) into a 200 ml beaker.

2. Homogenize the fluid for one minute.

3. Remove 1 ml of the fluid for dilution series (plate 0.1 ml of selected dilution on selective growth media (Plate Count Media, R2A, etc.)

4. Incubate for 2 to 7 days at 20oC (or desired temperature.).

5. Count plates containing 30-300 colonies.

6. Calculate surface cell densities from the plate count data. The surface area of the slides is determined by calculating: [(0.580 -2 x 0.06) x 5.90in D = 3.30in^2 or 18.75cm^2 or 1.875 x 10-3 m^2. Surface density calculations should be based on the biofilm scraped into a 100 ml volume, and sequential dilutions performed on the 100 ml solution.

10.3 Plating Protocol

Bacteria can be plate counted on selective agar or standard plate count agar (i.e., HPC or R2A; Standard Methods for the Evaluation of Water and Wastewater, 1989, 17th Edition, American Public Health As- sociation, Port City Press, Baltimore, MD).

As a first approximation for plating, a mono layer surface coverage generally is on the order of 1010 to 1011 cells/m2. A thin biofilm often contains 1011 to 1012 cells/m2 and a thick biofilm on the order of 1012 to 1014 cells/m2. When plating, several dilutions should be plated in triplicate to ensure valid plate count data is obtained. Often a dilution above and below the suspected bacterial dilution that will provide 30- 300 cell colonies/plate, are plated. If no bacterial polymer/slime suspension is observed during scraping, the cell count will likely be less than 1010 cells/ m2.

Appendix A – Reference Literature

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Appendix B – Biofilm Annular Reactor Parts List

BAR 1420 LJ - Laboratory Jacketed Biofilm Annular Reactor

Top Plate Assembly with Bearing and Shaft Seals

- Polycarbonate Top Plate (BST 1118 A) 1
- Aluminum Ring Plate Stiffener (BST 702) 1
- TN21 Dixon Hose Barb 7
- O-ring, Glass Cylinder (BST 2-362N70) 1
- O-ring, Water Jacket (BST 2-369N70) 1
- O-ring, Hole Plug (BST 3-914N70) 1
- Hole Plug (BST 115) 1
- Bearing and Seal Housing (BST 1107 A) 1
- Bearing (BST R-8ZZST) 1
- Shaft Seals (BST 4935) 2
- Motor Mount Plate (BST 1103 A) 1
- Cap Head Hex Screws (Stainless Steel) 10-32 x 3/4 4
- Flat Head Hex Screws (Stainless Steel) 10-32 x 5/8 4 4

Bottom Plate Assembly

- Polycarbonate Bottom Plate (BST 1119 A) 1
- Aluminum Ring Plate Stiffener (BST 1122 A) 1
- T33 Elbow Hose Barb 2
- O-ring, Glass Cylinder (BST 2-362N70) 1
- O-ring, Water Jacket (BST 2-369N70) 1
- Ball Tower (BST 1110) 1
- Pivot Bushing (BST 1111) 1
- Flat Head Hex Screws (Stainless Steel) 10-32 x ¾ 4

Inner Cylinder Assembly

- Inner Cylinder (BST 1106) 1
- Slide Coupons (see Section 7.0 for materials) (BST 503) 20
- Ball Bearing (Stainless Steel) (BST 9529K15) 1

Reactor Assembly

- Reactor Feet (1/4-20 x 1 plastic head screws) 3
- Stand-offs (stainless steel with Buna Cover) 4
- Brass Knurled Nuts (1/4-20) 8
- Tubing Support/Stand (BST 1150) 1

Motor Assembly

- Motor / Motor Controller Box (BAR 1420MC-1) 1
- Power Cord / Adapter 1
- 5 AMP Fuse 1
- 500 mAMP Fuse 1

BAR 1420 LS - Laboratory Biofilm Annular Reactor

Top Plate Assembly with Bearing and Shaft Seals

- Polycarbonate Top Plate (BST 1108 A) 1
- Aluminum Ring Plate Stiffener (BST 701) 1
- TN21 Dixon Hose Barb 1
- O-ring, Glass Cylinder (BST 2-362N70) 1
- O-ring, Hole Plug (BST 3-914N70) 1
- Hole Plug (BST 115) 1
- Bearing and Seal Housing (BST 1107 A) 1
- Bearing (BST R-8ZZST) 1
- Shaft Seals (BST 4935) 2
- Motor Mount Plate (BST 1103 A) 1
- Cap Head Hex Screws (Stainless Steel) 10-32 x 3/4 4
- Flat Head Hex Screws (Stainless Steel) 10-32 x 5/8 Shaft 4
- Coupler -6428K41 zinc hub 2
- Coupling Neoprene Spider/Coupler -6428K51 1

Bottom Plate Assembly

- Polycarbonate Bottom Plate (BST 1109) 1
- Aluminum Ring Plate Stiffener (BST 1112) 1
- T33 Elbow Hose Barb 2
- O-ring, Glass Cylinder (BST2-362N70) 1
- Ball Tower (BST 1110) 1
- Pivot Bushing (1111) 1
- Flat Head Hex Screws (Stainless Steel) 10-32 x 3/4 4
- Glass Outer Cylinder (BST 511-1 B) 1

Motor Assembly

- Motor / Motor Controller Box (BAR 1420MC-1) 1
- Power Cord / Adapter 1
- 5 AMP Fuse 1
- 500 mAMP Fuse 1

Inner Cylinder Assembly

- Inner Cylinder (BST 1106) 1
- Slide Coupons (see Section 7.0 for materials) (BST 503) 20
- Ball Bearing (Stainless Steel) (BST 9529K15) 1

Reactor Assembly

- Reactor Feet (1/4-20 x 1 plastic head screws) 3
- Stand-offs (stainless steel with Buna Cover) 4
- Brass Knurled Nuts (1/4-20) 8
- Tubing Support/Stand (BST 1150) 1

Appendix C – Reactor Speed / Pipe Flow Calculations

Operating Speeds (RPM) for the Model 1420 Biofilm Annular Reactor Hazen-Williams Coefficient = **100**								
Velocity (ft/sec)	**1**	**1.25**	**1.5**	**1.75**	**2**	**2.5**	**3**	**3.5**
Pipe Diameter (Inches)								
4	134	185	241	301	364	502	653	815
6	127	176	228	285	345	476	619	772
8	122	169	220	274	333	458	596	743
10	119	164	213	266	323	445	578	722
12	116	160	208	260	315	434	565	705
14	114	157	204	255	309	426	553	690
16	112	154	201	250	303	418	544	678
18	110	152	197	246	299	412	535	668
20	108	150	195	243	295	406	528	659
24	106	146	190	237	288	396	515	643
30	103	142	185	230	279	385	500	624
36	100	138	180	225	273	376	488	610
42	98	136	176	220	267	368	479	597
48	97	133	173	216	262	362	470	587

Operating Speeds (RPM) for the Model 1420 Biofilm Annular Reactor Hazen-Williams Coefficient = **110**								
Velocity (ft/sec)	**1**	**1.25**	**1.5**	**1.75**	**2**	**2.5**	**3**	**3.5**
Pipe Diameter (Inches)								
4	117	161	210	262	318	438	569	710
6	111	153	199	249	301	415	540	673
8	107	147	192	239	290	400	519	648
10	104	143	186	232	281	388	504	629
12	101	140	182	227	275	379	492	614
14	99	137	178	222	269	371	482	602
16	97	134	175	218	265	365	474	592
18	96	132	172	215	260	359	467	582
20	94	130	170	212	257	354	460	574
24	92	127	166	207	251	346	449	561
30	89	124	161	201	243	336	436	544
36	87	121	157	196	238	328	426	531
42	86	118	154	192	233	321	417	521
48	84	116	151	189	229	315	410	512

Operating Speeds (RPM) for the Model 1420 Biofilm Annular Reactor Hazen-Williams Coefficient = **120**								
Velocity (ft/sec)	**1**	**1.25**	**1.5**	**1.75**	**2**	**2.5**	**3**	**3.5**
Pipe Diameter (Inches)								
4	103	142	185	231	280	386	502	627
6	98	135	176	219	266	366	476	594
8	94	130	169	211	256	353	458	572
10	91	126	164	205	248	342	445	555
12	89	123	160	200	242	334	434	542
14	87	121	157	196	238	328	426	531
16	86	118	154	193	233	322	418	522
18	84	117	152	190	230	317	412	514
20	83	115	150	187	227	312	406	507
24	81	112	146	182	221	305	396	495
30	79	109	142	177	215	296	385	480
36	77	106	138	173	210	289	376	469
42	75	104	136	169	205	283	368	460
48	74	102	133	166	202	278	362	452

Operating Speeds (RPM) for the Model 1420 Biofilm Annular Reactor Hazen-Williams Coefficient = **130**								
Velocity (ft/sec)	**1**	**1.25**	**1.5**	**1.75**	**2**	**2.5**	**3**	**3.5**
Pipe Diameter (Inches)								
4	92	127	165	206	250	344	448	559
6	87	120	156	195	237	326	424	530
8	84	116	151	188	228	314	409	510
10	81	112	146	183	221	305	397	495
12	79	110	143	178	216	298	387	483
14	78	107	140	175	212	292	379	474
16	76	106	137	172	208	287	373	465
18	75	104	135	169	205	282	367	458
20	74	102	133	167	202	278	362	452
24	72	100	130	163	197	272	353	441
30	70	97	126	158	191	264	343	428
36	69	95	123	154	187	258	335	418
42	67	93	121	151	183	252	328	410
48	66	91	119	148	180	248	322	402

Appendix D – Reactor Surface Area and Liquid Volume

Surface Area Calculation:

Model 1420 Biofilm Annular Reactor

Superficial Area

Top Plate:	D=15.6 cm	piD^2/4	**226.97**		
Bottom Plate	D=15.6 cm	piD^2/4	**226.97**		
inner cylinder					
top	D=5.5 in;13.97cm			piD^2/4	148.21
bottom	D=5.5 in;13.97cm			piD^2/4	148.21
side	D=5.5 in;13.9	H=5.75		piDH	640.97
draft tubes	D=0.5 inc;1.2	H=9.05-3.15 in		piDH	239.16
	inner cyl,. Surface area		**1176.54**		
Glass cylinder	D=15.6 cm	H=7.125in;18.0975 cm		piDH	**886.91**

nominal surface area **Total** **2517.40** cm^2 (includes surface area of slides)
(not complete wetted)

=~2520 cm^2

Slide Surface Area

	in	cm				
H=5.90 inches		14.986				
W=0.580 inches-beveled edg	0.460	1.168				
Per Slide:			area=	**17.51** cm^2	Volume=	**2.80**

AR Working Volume: **1.0 Liters** (Standard Inner Cylinder; measured)