BioSurface Technologies Corporation

Industrial Surfaces Biofilm Reactor (ISBR 600) Operator's Manual

Designed in conjunction with the Center for Biofilm Engineering, Standardized Biofilm Methods Laboratory for BioSurface Technologies Corporation

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The Industrial Surfaces Biofilm Reactor (ISBR) is a bench-top biofilm growth chamber designed to provide a growth environment for biofilm under low shear, high gas transfer, and intermittent wetting. The sample slides are intermittently wetted via a drip or spray port in the lid of the reactor. The nutrient stream gravity flows into a sump located at the bottom of the reactor and may be recycled or drained to waste. This reactor environment is similar to those in industrial cooling towers, food preparation surfaces, painted and coated surface testing, marine environments, and other periodically wetted or humid biofilm growth surfaces. The ISBR can be used to model biofilm growth found in a large variety of industrial applications and public health environments.

1. Biofilm Reactor

The components of this reactor include a multi-ported glass vessel, a ported lid, a lid seal assembly, a coupon holder disc, and 18 slide coupons (various materials available). The reactor components are autoclavable and re-useable. The slide coupons are mounted in the coupon holder disc vertically and are rotated under the nutrient stream via a stir plate. The slide mounting in the disc allows for several different mounting angles (range of 80 degrees to 100 degrees from the disc) to allow the nutrient flow to preferentially contact the front, back, or both sides of the slide coupon. Each slide is approximately 0.6 inches x 3.0 inches (15.25 mm x 76.2 mm). The slides are held in place in the coupon holder disc by a silicone rubber disc with slots which maintains the slide angle and support during assembly and operation.

2. Coupons

The reactor is provided with a set of polycarbonate slide coupons that are autoclavable and re-useable. Each slide is approximately 0.6 inches x 3.0 inches (15.25 mm x 76.2 mm) and 1mm thick (0.039 inches, +/- 0.005 inches). A variety of slide materials are available including stainless steels, glass, and plastics. Each slide is inserted into a slot on the slide holder disc. The holder has a silicone rubber disc with slots that hold each slide in place.

3. Reactor Assembly

The reactor requires assembly prior to each use.

A. Slide Coupon Assembly

The coupon holder disc consists of four (4) stacked layers of polycarbonate and silicone rubber discs, held together by four (4) 316 stainless steel screws. The top polycarbonate and silicone discs may be rotated relative to the lower assembly to allow a range of slide coupon angle (range of about 80 to 100 degrees from horizontal; angle may vary due to coupon thickness and material) relative to the coupon holder disc base. The ISBR is provided with the angle at about 80 degrees. To assemble the reactor, the coupon holder disc is removed from the reactor and slides inserted into the holder slots. The silicone disc helps holds each coupon in place. Once the desired coupons are in place on the disc, the disc assembly is carefully placed into the reactor vessel.

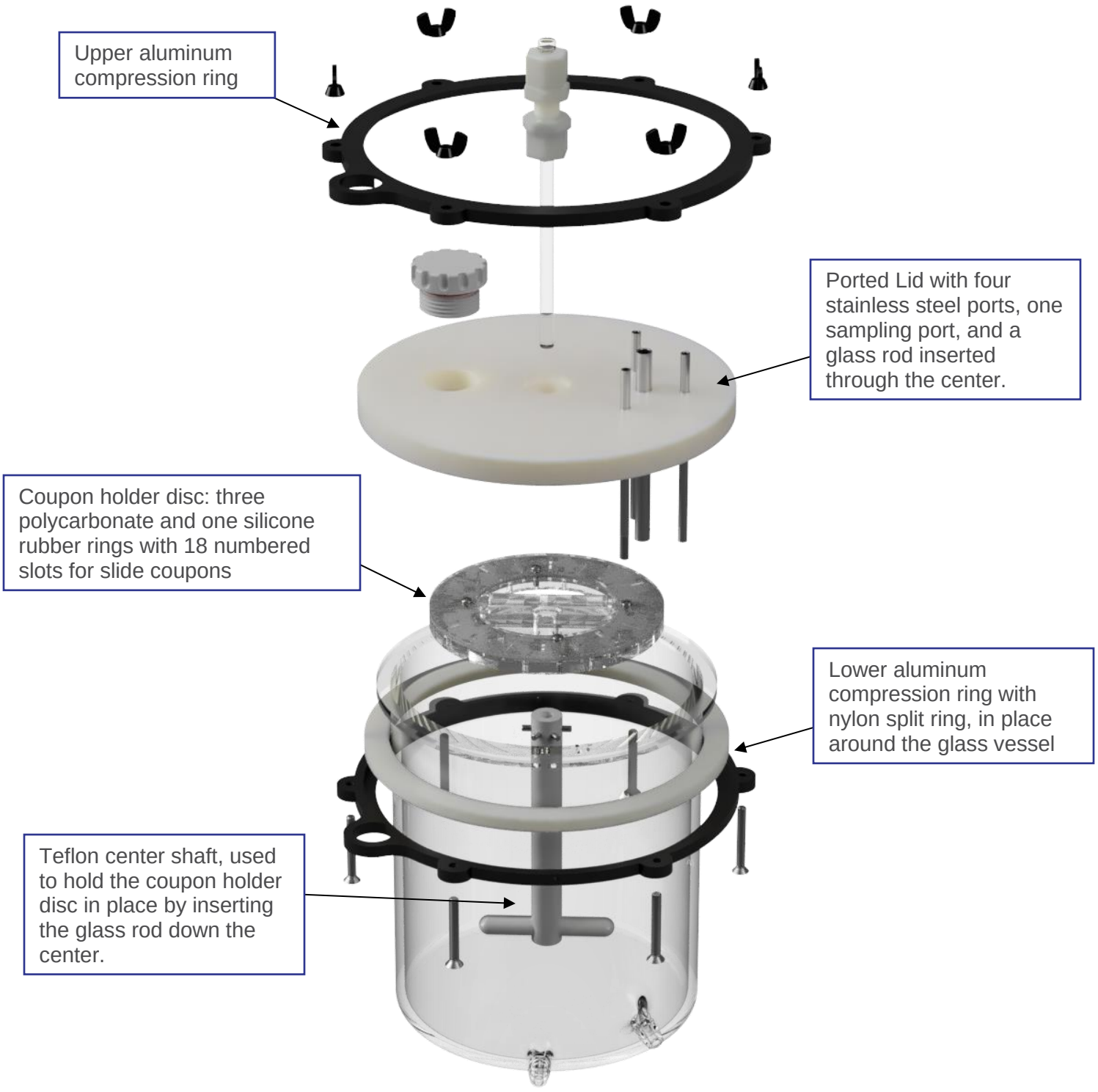

Figure 1: Exploded view of the Industrial Surfaces Biofilm Reactor.

NOTE 1: Overtightening of the screws in the coupon holder disc my result in improper fit of the slides. The screws should be tightened just enough to hold the coupon holder disc together, but not enough to warp the silicone layer.

B. Lid Assembly

A glass shaft connected to the lid is inserted into the Teflon shaft on the coupon holder disc while it is in the reactor. The lid is then oriented over the glass lip on the glass vessel. An O-ring embedded in the lid seals to the glass vessel with the aid of the aluminum compression rings.

C. Lid Seal Assembly

The lower aluminum compression ring is placed over the glass vessel prior to installing the reactor lid with the screw threads facing up. A two-piece nylon flange is installed in the top side of this lower aluminum compression ring. Align the two screw holes in the edge of the nylon flange pieces with the holes through the side of the lower aluminum compression ring. Two screws are used to hold these nylon flange pieces to the lower aluminum compression ring. The upper aluminum compression ring is placed over the reactor lid. The lower compression ring is raised and the six threaded screws on the lower compression inserted through the six holes on the upper compression ring. The lid seals to the glass vessel by tightening the black nylon wingnuts. Do not overtighten the wingnuts (wingnuts should be finger tight). The lower compression ring and nylon flange, once installed, can remain on the reactor during cleaning and storage.

D. Reactor Mounting on Stir Plate

Once the coupons, coupon holder disc, lid, and compression rings have been installed, the reactor is ready for operation on a stir plate. The assembled ISBR requires mounting on magnetic stir plate to rotate the coupon holder disc under the influent stream port in the lid. The reactor should be centered on the stir plate. A large hole on the top aluminum compression ring is used to slide over the supplied chem stand support rod mounted on the stir plate. The chem stand support rod will aide in stabilizing the reactor and keep the reactor from tipping or falling off the stir plate.

NOTE 2: The ISBR requires a stir plate with very strong magnets to link the stir plate with the magnet on the coupon holder disc. The ISBR is provided with a stir plate designed with strong magnetic linkage for mixing large liquid volumes. Standard laboratory stir plates may not provide the magnetic linkage required to rotate the coupon holder disc.

E. Reactor Recycle and Nutrient Supply Lines

The ISBR glass vessel includes two side discharge ports. The lower of the discharge ports may be used to pull liquid from below the coupon holder disc for recycle flow. The upper discharge port is used for effluent flow from the system into a waste collection reservoir.

> ***Reactor Recycle:*** The ISBR was designed for nutrient recycle via the bottom side discharge port. A separate recycle pump (not included with the standard kit) is used to recycle liquid from the reactor basin to the influent port. Recycle rates are set depending on the environmental or industrial system to be modeled (i.e., cooling tower has high recycle flow, while paint, surface coatings, and other intermittently wetted surfaces may use lower recycle flow rates).

Nutrient Supply: The ISBR lid is provided with four ports. One port is commonly used as a vent port or specialty gas supply port (generally the larger diameter port). Nutrient addition is provided through one of the other three ports. Two of the ports are situated directly above the center of the slide coupons when installed in the coupon holder disc. One of these two ports is provided with external 10-32 threads that allow mounting of drip or spray nozzles (not included; contact BioSurface Technologies for further information) if desired. If reactor recycle is to be used, the nutrient supply line can be tee'd into the recycle line just prior to the nutrient supply port. A separate nutrient supply pump (not included with the standard kit) is used to provide nutrient media to the reactor. The fourth port in the reactor lid may be used to introduce chemical treatments or alternative nutrient supply directly to the sump media at the bottom of the vessel due to its placement outside the diameter of the coupon holder disc. Ports not in use should be clamped off using a tubing clamp, to avoid contamination during experiments.

4. Autoclaving Instructions

The ISBR is autoclavable and re-useable (some slide materials may not be autoclavable; check compatibility prior to autoclaving and during experimental design planning stages).

To autoclave the ISBR, place the assembled reactor vessel (never autoclave the stir/hot plate or expose it to water or steam) into a plastic autoclave bin. Cap open tubing ends with aluminum foil, or autoclave paper. Loosen nylon wingnuts holding the compression rings in place (do not remove; loosen 1-2 turns to prevent damage during autoclaving and to allow for expansion). Autoclave for 15 to 20 minutes at 121°C.

NOTE 3: Autoclaving longer than 20 minutes could cause unnecessary degradation to the reactor or its components and is not advised. The reactor and its components have not been approved for temperatures above 121°C, and any damage caused by a higher temperature will not be covered under warranty.

5. Safety

- The ISBR mechanical fittings should be loosened prior to autoclaving to prevent stress to the reactor and to allow for differential expansion.
- The ISBR glass vessel is not designed to operate under positive pressure. The reactor vessel and waste collection vessel should always remain vented to the atmosphere.
- The ISBR should be operated in a containment vent hood when introducing hazardous cultures or chemicals to the reactor system.
- Use of a spray nozzle for liquid introduction into the ISBR may produce hazardous aerosols. Containment and a suitable aerosol settling period should be utilized when operating with a spray nozzle.

NOTE 4: The operator of this reactor system should always consult with the laboratory safety officer prior to use and follow all necessary safety procedures and protocols. BioSurface Technologies Corporation is not responsible for any illness or injury caused while using this reactor system.

5. Standard Operating Procedure

Method developed using BAC water. Operator may want to alter the method to suit their needs, laboratory setup, and bacteria type.

A. Material Preparation

- *R2A Agar Plates:* Prepare 2-3 days before use.
- *Dilution Tubes:* Sterile. Filled with 9mL sterile dilution water.
- *Hemostats.* Minimum 6 inches in length.
- *Rinse Tubes:* Fill sterile 50 mL centrifuge tubes with 45 mL of sterile dilution water, used to rinse individual coupons.
- *50-mL Centrifuge Tubes:* Fill with 40 mL of sterile dilution water, for coupon drop method.
- *Pumps for Continuous Feed and Recycle Stream:* Capable of flow rates ranging between 0.5 - 80 mL/min.
- *Autoclavable container capable of holding a minimum of 3 liters for Continuous Feed and Effluent Streams.*
- *Stir Plate:* 30 to 45 rpm is suggested.

NOTE 5: While the stir plate can be adjusted from 1 to 1000+ rpm, it cannot rotate the coupon holder disc at these speeds. The suggested operating rotational speed is 30 to 45 rpm and should not exceed 75. When choosing a rotational speed, make sure to check that the coupons remain vertical at the chosen speed. If the speed is too fast, the coupons may tilt or fall out. Additionally, if the stir plate does not bring the coupon holder disc up to the set speed within a minute or two, turn it off and restart. It may take several tries before the stir plate is able to hold the disc at the set speed. If the disc is not coming up to speed, flame sterilize a set of forceps and push the small pegs at the top of the Teflon center shaft to start the rotation. Do not touch anything but the pegs.

B. Nutrient Broth Preparation

- *Batch Broth:* 300 mg/L TSB. Dissolve 0.09 g of Tryptic Soy Broth in 100 mL of sterile deionized water and autoclave. Add this to 200 mL of BAC water. Do NOT sterilize BAC/TSB water.
- *Continuous Flow Broth:* 100 mg/L TSB final concentration. Confirm volume in carboy after autoclaving and prepare and sterilize a TSB solution to add to obtain a final concentration of 100 mg/L in the carboy.

C. General Instructions

a. Day One: Batch Phase

1. Assemble reactor components and tubing.
2. Place the complete reactor system in an autoclave tray and autoclave on “dry” setting with fast exhaust. Let cool after autoclaving before proceeding.
3. Sample 30 mL BAC water and plate using the spread plate method (SOP *SBM 8: Spread Plate Method)*. Plate in triplicate. This will be used to determine baseline growth in the reactor. Incubate at room temperature for 7 days.
4. Calibrate the recycle stream pump to 55 ml/min.
5. Clamp effluent stream tubing to prevent loss of batch broth. Connect the effluent stream tubing to the waste carboy.
6. Add the Batch Broth to the reactor.
7. Center reactor on stir plate and set to 30 rpm.
    a. If required, flame sterilize forceps and push the center rod using the small metal pegs at the top to help begin the rotations.
8. Once the disc is rotating, turn on the recycle stream pump to start the flow of recycled Batch broth.
9. Ensure no leaks and/or spills are occurring.
10. Ensure the recycle stream is flowing into the reactor from the influent port in the top of the reactor.
11. Calibrate the influent stream pump to pump at 2 mL/min. Set aside for the next day.
12. Allow this configuration to run constantly for 24 hours at room temperature.

b. Day Two: Continuous Phase

1. Attach the influent stream tubing to the Continuous Flow carboy. Turn on the pump to begin flow from the influent stream.
2. When the Continuous Flow nutrient broth reaches the Batch Broth, unclamp the Effluent Stream port and allow the excess Batch Broth to drain to the waste carboy.
3. Run this configuration continuously for 24 hours at room temperature.

c. Day Three – Sampling (Modified Single Tube Method)

1. Turn off the stir plate and 2 pumps. Allow aerosols to settle for 30 minutes prior to opening the reactor.
2. Using a serological pipet, remove 5 mL of reactor broth, dilute and spread plate in triplicate.
3. Flame-sterilize hemostats using 95% ethanol. (Repeat this before removing each coupon from the reactor and before replacing the coupon with another.)
4. Use the hemostats to retrieve one coupon from the reactor.
5. Rinse the coupon in a 45 mL rinse tube by gently immersing the coupon with a fluid motion and pulling it out.

6. Drop the coupon into a 50-mL centrifuge tube containing 40 mL sterile dilution water.
7. Use flame-sterilized hemostats to replace the extracted coupon with replacement coupon.
8. Vortex and sonicate the samples for a total of 5 times each lasting at least 30 seconds.
9. Serially dilute the samples. The dilutions should go to at least the 6th dilution for the reactor broth and the 5th dilution for the coupons.
10. Spread plate in triplicate.
11. Incubate plates at room temperature for 7 days before counting.

d. Day 10: Cell Enumeration

1. Count dilutions with 30 and 300 CFU per plate. Record the counts and the corresponding dilution directly into the lab notebook. Calculate the log density according to equations in the following "Calculations" section.

D. Calculations

a) Calculate the log density for one coupon using Equation [1]:

$$LOG_{10}\left(\frac{cfu}{cm^2}\right) = LOG_{10}\left[\left(\frac{\left(mean\ cfu \big/ plate\right)}{vol.of\ sample\ plated}\right) \times \left(\frac{volume scraped\ into}{surface area\ scraped}\right) \times \left(dilution\right)\right] \quad \textbf{[1]}$$

Or

LOG_{10} (CFU/cm^2) = LOG_{10} [(X/B) (V/A) (D)]
X = mean CFU
B = volume plated
V = volume scraped into
A = surface area scraped (2.5 cm x 7.5 cm)
D = dilution

Example: X (mean CFU) = 100
B (Volume plated) = 0.1 mL
V (Volume scraped into) = 50 mL
A (Microscope slide surface area) = 37.5 cm^2
D (dilution) = 10^5
LOG_{10}(CFU/cm^2) = LOG_{10}[(100/0.1) (50/37.5) (10^5)] = 8.12

E. Clean Up

1. Do NOT empty contents of reactor until after autoclaving is complete.
2. Drain influent, effluent and recycle tubing into the reactor.
3. Clamp the effluent stream tubing.
4. Remove influent and effluent tubing from their respective carboys, cover with aluminum foil, and secure with autoclave tape.
5. Add a volume of bleach to the waste carboy to be 10% of the total volume contained in the carboy. Allow to stand for at least 24 hours.
6. Pour the rest of the sterile continuous feed broth into a sink.

7. Rinse the continuous flow carboy 3 times with tap water, and 3 times with deionized water.
8. Place the reactor and any other waste in an autoclave tray and autoclave on “liquid” setting for 30 minutes, slow exhaust.
9. After autoclaving is complete and the reactor is cool, carefully empty the reactor into a sink and clean all components with 10% Micro90 solution.
10. Rinse the reactor 3 times with tap water, and 3 times with deionized water.
11. Let all components air dry.
12. Empty the waste carboy into a sink 24 hours after adding the bleach.
13. Inspect the influent and recycle stream tubing for wear. Replace tubing when necessary. The recycle stream requires a high rate of flow so this tubing will become damaged more quickly than the influent stream tubing.
14. Once the components are done drying, reassemble the reactor in preparation for its next use.

Notes:

P. aeruginosa was tested as opposed to BAC. A plate was streaked and incubated in the incubator for 24 hours, then incubation broth was inoculated and put in shaker for 24 hours at 125 rpm and 300 mg/L. The inoculum was spread plated, but again incubated in the incubator for 24 hours, not at room temp for 7 days.

For sampling, the samples were drop plated, not spread plated. They were also put in the incubator for 24 hours.

Equation used in data sheet for CFU/cm^2 and $\log_{10}$(CFU/cm^2):

$$\frac{CFU}{cm^2} = \frac{averageCFU}{volume\ plated * 10^{-dilution}} * \frac{total\ volume}{coupon\ surface\ area}$$

$$Log_{10}\left(\frac{CFU}{cm^2}\right)$$

6. Do Not Pressurize or Operate Under Vacuum

The Industrial Surfaces Biofilm Reactor sealed lid for containment does not make the Industrial Surfaces Biofilm Reactor a pressure or vacuum containment vessel. The seal added to the lid is to aid in containment of gases and particles. The Industrial Surfaces Biofilm Reactor vessel should always be properly vented.

BioSurface Technologies does not recommend autoclaving with the lid clamps in place, nor is this necessary. All components of the Industrial Surfaces Biofilm Reactor (ISBR 600) are autoclavable and re-useable.