BioSurface Technologies Corp.

CDC Biofilm Reactor (CBR)

Operator's Manual

Prepared by the Center for Biofilm Engineering, Standardized Biofilm Methods Team
For BioSurface Technologies, Inc. Last Updated: May 2016.

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ASTM standard methods for the use of the CDC Biofilm Reactor (www.astm.org):

ASTM E2562-12: Standard Test Method for Quantification of *Pseudomonas aeruginosa* Biofilm Grown with High Shear and Continuous Flow using CDC Biofilm Reactor

ASTM E2871-13: Standard Test Method for Evaluating Disinfectant Efficacy against Pseudomonas aeruginosa Biofilm Grown in CDC Biofilm Reactor using Single Tube Method

1. Purpose

The CDC Biofilm Reactor Operator's Manual is intended to serve as a guide for researchers interested in growing a laboratory biofilm under high shear conditions. It is the responsibility of the user to be familiar with basic microbiological concepts and techniques. Although a specific method is presented in the manual, the CDC Biofilm Reactor is suitable for modeling many different environments. Laboratory biofilms are engineered based upon the dynamics in the reactor where they grow. No one biofilm is better than any other, although one reactor may be a better choice for modeling a particular environment. It is the responsibility of the operator to choose which reactor best suits their research needs.

2. CDC Biofilm Reactor Description

The CDC Biofilm Reactor (CBR), Figure 1, is a one-liter vessel with an effluent spout at approximately 400 ml. Continuous mixing of the reactor's bulk fluid is provided by a baffled stir bar that is magnetically driven. An UHMW polyethylene top supports eight independent rods. Each rod houses three removable coupons (biofilm growth surfaces) for a total of 24 sampling opportunities. The coupons may be constructed from a variety of materials, for example: polycarbonate, porcelain, mild steel, stainless steel, PVC, vinyl, glass, etc. The coupons experience a consistent high shear from the rotation of the baffled stir bar. A researcher may vary shear by altering the baffle's rotational speed. The CBR operates as a continuous flow stirred tank reactor (CFSTR), meaning nutrients are continuously pumped into and flow out of the reactor at the same rate. A CFSTR offers the following advantages:

- the bulk fluid (all the fluid in the reactor) is assumed to be well mixed (no gradients);
- the surfaces within the reactor experience fluid shear (force opposing flow) from the mixing;
- the reactor can achieve steady state conditions which eliminates all terms that depend upon time in the mathematical representation of the reactor. This simplifies the mathematics necessary to calculate the reduction in biofilm accumulation as a result of a treatment;
- the residence time (time for one reactor sized volume of liquid to flow through the reactor) in the reactor is easily adjusted to select for biofilm growth for various species of bacteria;
- the nutrient feed may be specified and adapted for various bacteria or conditions (for example to represent a swimming pool environment or the white water associated with the paper industry);
- a portion of the reactor's effluent (waste) may be recycled back into the reactor or become the feed of a second reactor;
- with simple modifications, a CSFTR can be operated anaerobically (without oxygen). This increases the number of relevant environments that can be modeled using this design.

In addition to the list of advantages above, the CBR offers additional advantages:

- the biofilm growth surfaces (a.k.a. coupons) are interchangeable with a current reactor that BioSurface Technology manufactures and sells;
- 24 sampling opportunities;
- removable rods that allow for intermittent removal; control and treated data from the same reactor; and
- a baffled stirrer provides for consistent shear across all the coupons.

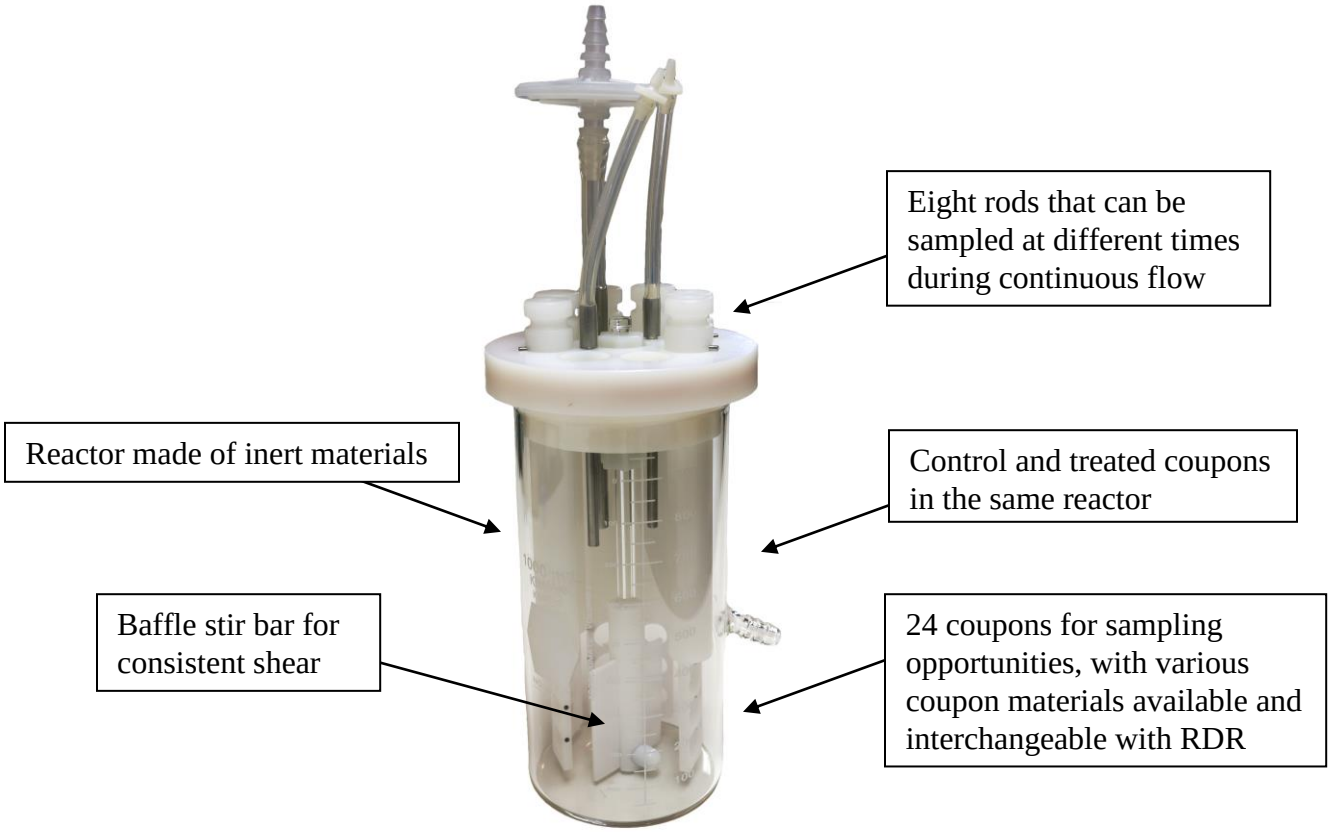

Figure 1. Picture of the CDC Biofilm Reactor.

3. CBR Standard Operating Procedure

The CBR was designed as a flexible reactor system, meaning that it can be easily adapted to model a variety of conditions in the laboratory. The following standard operating procedure (SOP) is just one suggested use. Operators are encouraged to modify the protocol to model the most appropriate biofilm for their research needs. A series of notes are included in the SOP that alert the operator to situations where special care must be taken.

Caution: All microorganisms should be handled according to safety recommendations for each individual species of microorganism. Decontamination of all media and equipment used during experimentation is required prior to disposal of media or re-use of equipment. It is the responsibility of the operator to inform themselves on these techniques.

A. Principle

Biofilm is defined as an accumulation of bacterial cells immobilized on a substratum and embedded in an organic polymer matrix of microbial origin. Biofilm bacteria exhibit a different phenotype from suspended bacterial cells of the same genotype. In this method, a laboratory biofilm is grown under high shear and continuous flow conditions. Biofilm accumulation is quantified by harvesting the biofilm from coupons of a known surface area, disaggregating the cells and polymer matrix and performing viable plate counts.

B. Apparatus, Figure 2

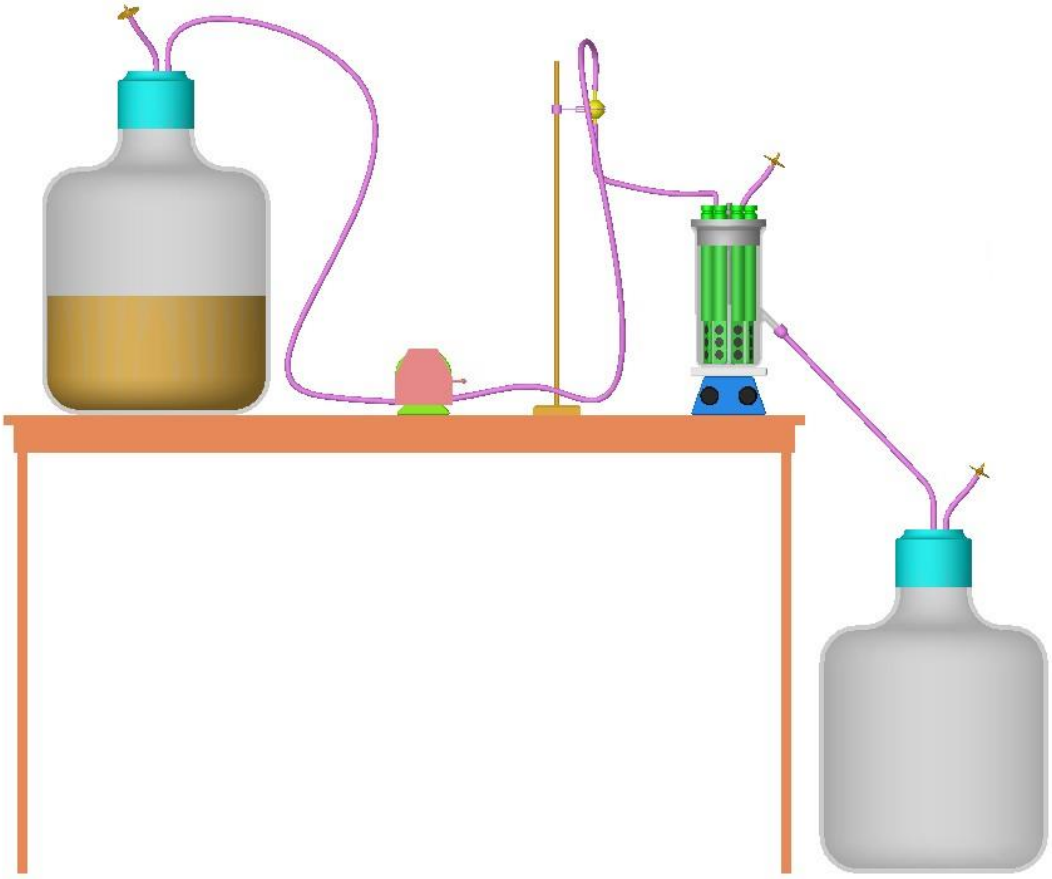

Figure 2. Schematic of the completely assembled reactor system (Norris, 2003).

(a) Reactor Components

i) Berzelius Pyrex Beaker: 1000mL beaker without conventional pour spout, 9.5 +/- 0.5 cm diameter. Pyrex barbed outlet spout added so that bottom is at 400 mL +/- 20mL mark. Angle the spout 30-45 degrees to ensure drainage. Spout should accommodate silicon tubing with ID of 8-11 mm.

NOTE 1: The rods (B.a.iii) and baffle (B.a.v) will displace approximately 50 mL of liquid when the system is completely assembled. Therefore, an outlet spout at the 400 mL mark will result in a volume of approximately 350 mL during operation. Before beginning an experiment, confirm the actual reactor operating volume when the reactor is fully assembled.

ii) Reactor top, Figure 2: UHMW polyethylene top equipped with 3 holes accommodating 6-8 cm long pieces of stainless steel or other rigid autoclavable tubing with OD of 5-8 mm for media inlet, air exchange and inoculation port. Center hole, to accommodate the glass tubing used to support the baffle assembly (B.a.v). Eight rod holes, notched to accommodate stainless steel rod alignment spike. Rubber gasket, to fit between reactor top and reactor beaker.

Figure 2. Expanded schematic of reactor top (Norris, 2003).

iii) Polypropylene rods, Figure 3: Eight polypropylene rods, machined to hold three coupons (B.a.iv) at the immersed end. 316 Stainless Steel set screws imbedded in side to hold coupons in place. Rods fit into holes in reactor top and lock into preformed notches.

iv) Polycarbonate coupons, Figure 3: Provide a surface for biofilm growth. Twenty-four cylindrical polycarbonate coupons with a diameter of 1.27 cm, thickness of approximately 3.0 mm.

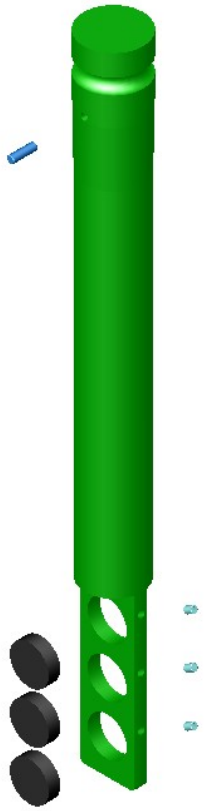

Figure 3. Expanded schematic of coupon rod and coupons (Norris, 2003).

v) Stir Blade Assembly (Baffle), Figure 4: To create constant shear/mixing. Teflon blade) fitted into cylindrical Teflon holder and held in place with a magnetic stir bar. Teflon holder fits onto a glass rod, Figure 2, fitted into the reactor top. The glass rod is held in place with a compression bulk-head fitting and acts as a support for the moving blade assembly.

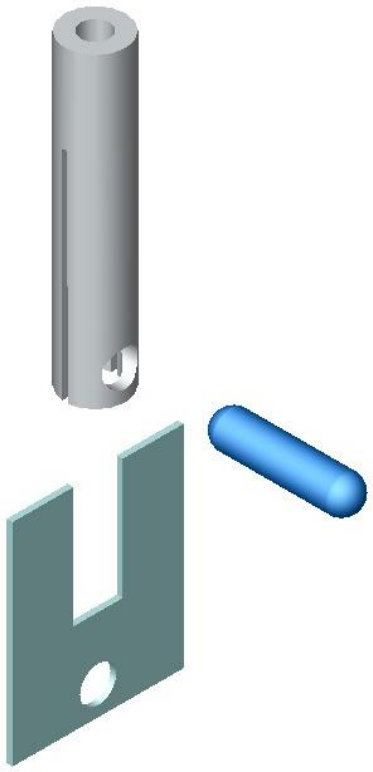

Figure 4. Expanded schematic of baffled stir bar (Norris, 2003).

(b) Glass Flow break: Autoclavable and accommodating tubing of ID 3.2 mm. Insert vertically into media inlet tube to prevent contamination from reactor to media carboy.
(c) Silicone tubing: Two sizes of tubing, one with ID 3.2 mm (port OD=4.75 mm) and the other with ID 7.9 mm (port OD=7.94 mm). Both must withstand sterilization. Use to connect media carboy to reactor inlet; beaker outlet to waste carboy; air vent to reactor top; media carboy air vent.
(d) Carboy: Two 20L autoclavable carboys for media and waste.
(e) *Carboy Tops:* Two threaded tops each equipped with 2 barbed fittings to accommodate tubing ID 3.1 mm for nutrient or waste line and air vent attachment.
(f) Magnetic Stir plate: Capable of operation at 100-400 rpm. A digital stir plate is recommended.
(g) Pipet: Capable of accurately dispensing 1.0 mL.
(h) Micropipet: Capable of accurately dispensing 0.01 mL.
(i) Top Loading Balance: For weighing reagent ingredients; sensitive to 0.01 gm.
(j) Homogenizer Probe: Capable of mixing at 20,500 +/- 5000 rpm in a 5-10 mL volume and able to withstand autoclaving or other means of sterilization.
(k) Vortex: Any vortex mixer that will ensure proper mixing of culture tubes.
(l) Peristaltic Pump: For pumping media into reactor during continuous flow phase; pump head capable of holding selected tubing (accommodates tubing ID/OD).
(m) Wooden Applicator Sticks: Used for scraping biofilm from coupon surface. Sterile.
(n) Hemostat: Stainless steel hemostat clamp with curved tip to remove and hold coupon in place while scraping. Use after flame sterilizing.

(o) Inoculating Loop. Used to introduce bacteria into inoculum flask. Sterile
(p) Bunsen or Alcohol burner: For flame sterilizing inoculating loop and hemostat.
(q) Culture Tubes with caps: Any with a volume capability of 10 mL and diameter no less than 6 cm. Recommended size is 16 x 125mm borosilicate glass with threaded opening.
(r) Sterilizers: Any steam sterilizer capable of producing the conditions of sterilization.
(s) Colony Counter: To aid in counting plates.
(t) Clamp Stand and Clamp: To hold glass flow break and media inlet tube vertically above reactor top. Clamp with 0.5 cm minimum grip size.
(u) Petri Dish: 100 x 15mm, plastic, sterile, for transporting rods from reactor to work station.
(v) Plexiglas Board: Or other surface, used as a sampling platform on which to hold and scrape samples. Use after disinfecting with 70% EtOH.
(w) Environmental Shaker: Capable of maintaining temperature of 37˚C.

C. Media and Reagents

NOTE 2: All reference to water as diluent or reagent means distilled water or water of equal purity.

(a) Inoculum culture media: 100mL of 300mg/L soybean-casein digest media or an equivalent general bacterial growth medium. Tryptic soy broth is recommended. Sterilize for 20 minutes. Inoculate media with a single colony from a bacterial isolation plate using sterile inoculating loop. *Pseudomonas aeruginosa* (ATCC 700888) is the bacteria used in this method. Incubate at 35+/- 2˚C while shaking for 18-24 h.
(b) Batch culture media: 500mL of 300mg/L TSB. Sterilize in reactor for 20 minutes.
(c) Continuous flow media: 20L of 100mg/L TSB. Sterilize 20L of water then aseptically add concentrated broth (2.0 gm TSB/500mL, sterilized 20 minutes) so that the final volume equals 20 L and final concentration equals 100 mg/L.
(d) Buffered Dilution Water: 0.0425 g/L KH_2PO_4 distilled water, filter sterilized and 0.405 g/L $MgCl \cdot 6H_2O$ distilled water, filter sterilized. Aseptically fill sterile, capped test tubes with 9 mL of sterile dilution water.
(e) Bacterial Plating Medium: R2A agar is recommended.
(f) 95% Ethanol: For dipping hemostat prior to flame sterilizing.
(g) 70% Ethanol: For rinsing homogenizer probe and general bench top clean up.

D. General Instructions

(a) Reactor Preparation

i) Clean reactor coupons by immersing in a 1:1000 dilution of laboratory soap and sonicating for 3-5 minutes. Rinse with tap water until no more soap appears then sonicate again in reagent grade water. A final 2 hour soak in 2M HCl is recommended then a final rinse with reagent water (do not soak hydroxy apatite or other acid susceptible coupons in HCl). Let coupons air dry. Place a coupon into each hole of the reactor rod, leaving the top of the coupon flush with the inside rod surface (sampled surface). Hold in place by tightening the set screw adjacent to the hole.

NOTE 3: Check for material compatibility before soaking coupons in 2 M HCl (hydroxy appatite, carbon steel, or stainless steel coupons should not be soaked in 2M HCl)

NOTE 4: Visually inspect coupon surface topography for any deformations before using. Discard damaged coupons.

ii) Assemble all reactor parts including rods, coupons, baffle, bacterial air vent and tubing. Air vent and tubing are attached to the rigid tubes in the reactor top (B.a.ii*).* Use a small piece of flexible tubing to attach the air vent to the reactor top. Check assembly on stir plate to ensure baffle rotates without scraping rods. Clamp effluent tube. Foil the exposed ends of all tubing.

iii) Add 500 mL non-sterile batch culture media (C.b). Sterilize reactor system for 20 minutes.

(b) Batch Phase

i) Place reactor on stir plate. Be sure gases may pass freely through the bacterial air vent and effluent tubing is clamped shut.

ii) Aseptically inoculate the reactor by injecting 1mL of previously prepared inoculum culture (C.a) into the batch media through an inoculum port in reactor top.

NOTE 5: The viable bacterial density of the inoculum should equal 10^8 cfu/mL. Confirm this number by diluting and plating a sample from the inoculum flask.

iii) Start the baffle rotating at 125 ± 5 rpm.

NOTE 6: The speed at which the baffle rotates directly determines the amount of shear stress that the biofilm experiences. Ruggedness testing showed that biofilm accumulation on the coupons is sensitive to changes in the baffle's rotational speed. ***The baffle rotational speed is a critical factor that must be controlled. If a digital stir plate is not available, then use a strobe light to confirm rotational speed.***

iv) Operate the reactor in batch phase for 24 hours.

(c) Continuous Flow Phase

i) Aseptically connect the nutrient tubing line to the carboy containing the continuous flow nutrient broth (C.c).

ii) Remove foil and place the end of the effluent tubing into a waste carboy, unclamp.

iii) Pump a continuous flow of media into the reactor at a flow rate equal 11.67 $\pm$ 0.2 ml/min.

NOTE 7: Flow rate is calculated by dividing the reactor volume by the residence time. The residence time is 30 minutes. The reactor volume is approximately 350 ml, see NOTE 1. The operator should set an exact flow rate based upon the measured fluid volume in the reactor when the baffle, rods, and coupons are in place to achieve an exact 30 minute residence time. The reactor residence time is a critical parameter that is specific to the bacterial species used during the experiment. In order to select for biofilm growth in the reactor, the residence time must be less than the doubling time for the suspended cells. This will result in the suspended cells washing out of the reactor, leaving only biofilm.

iv) The reactor is operated with continuous flow for 24 hours.

E. Sampling

(a) Sampling Coupons

i.) Set-up sampling materials: vortex, homogenizer, culture tubes, pipettes, wooden sticks, flame sterilized hemostat, sampling platform.

ii.) Turn off stir plate and pump.

iii.) Remove a single rod by pulling straight up. Collect any drips in a sterile petri plate held beneath rod.

iv.) Loosen set screw to release coupon and remove using flame sterilized hemostat, being careful not to disturb the biofilm on coupon surface that will be scraped.

v.) Hold coupon in place on a disinfected Plexiglas Board with hemostat and scrape the side of the coupon that faced the baffle with a sterile wooden stick. Hold the stick perpendicular to the surface when scraping.

vi.) Remove cap of first 9 mL dilution tube and rinse stick by swirling and tapping on bottom of tube. Repeat scraping and rinsing steps 3-4 times.

vii.) Hold coupon above dilution tube and rinse scraped surface with 1 mL dilution water. The total volume in the tube equals 10 mL. Record this number as the volume scraped into (Equation 1).

(b) Disaggregation

i.) Homogenize the dilution tube containing sample at 20,500 rpm for 30 s using sterile homogenizer probe.

ii.) Clean the probe between samples by rinsing for 30 s at 20,500 rpm in a sterile dilution blank followed by a rinse at 20,500 rpm for 15 s in 75% ethanol. Let the probe soak in ethanol for 1 min. Rinse probe two more times with dilution blanks at 20,500 rpm for 30 s each.

NOTE 8: Homogenization breaks up biofilm clumps to form a homogeneous cell suspension. Improper disaggregation will result in an underestimation of viable cells present in the sample.

(c) Serial Dilution

i.) Serially dilute the disaggregated biofilm sample according to standard microbiological techniques.

(d) Viable Colony Counts

i.) Plate each dilution in duplicate on R2A nutrient agar. Any standard plating technique, such as spread plating or drop plating, is acceptable.

ii.) Incubate plates at 35 +/- 2˚C for 18-24 hours.

iii.) Count the appropriate number of colonies for the plating method used.

F. Calculations

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

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

b) The mean biofilm density is the average of the individual coupon log densities.

4. Repeatability

For the CBR SOP, the estimated within-experiment variance is 0.1980 and the estimated between-experiment variance is 0.1461. The estimated repeatability standard deviation is 0.5866 of which 58% is attributable to within-experiment variation and 42% is attributable to between-experiment variation. This repeatability standard deviation pertains to a protocol that samples only one coupon per experiment. The repeatability standard deviation for a protocol that requires sampling n coupons per experiment is [(0.1980 / n) + 0.1461]–. For example, if the protocol specifies n=3 coupons and the reported log density is the mean of the three individual coupon log densities, then the repeatability standard deviation is 0.46 of which 31% is attributable to within-experiment variation and 69% is attributable to between-experiment variation.

5. Trouble Shooting

1. Always check the alignment of the rubber gasket, Figure 2.
2. Visually confirm that the inoculum reaches the reactor's bulk fluid.
3. The top of the glass rod is the end with the expanded head, Figure 2. This design holds the baffle in the center of the reactor while allowing it to rest on the bottom for proper mixing.
4. Tighten coupon set screws before the reactor is autoclaved to ensure correct alignment.
5. If a rod pops out of the reactor during autoclaving, immediately push it back into the reactor top and continue with experiment unless the operator suspects the reactor's sterility is compromised, then start over from the beginning of the protocol.
6. Always ensure that the reactor's effluent spout allows for proper draining.
7. If rod alignment peg falls out, Figure 3, replace before beginning an experiment.
8. Confirm that the bacterial air vent is working properly before an experiment begins, so that the reactor can exhaust during autoclaving.

6. CDC Biofilm Reactor Assembly

1.0 CDC Biofilm Reactor Assembly

Note 1: The CDC Biofilm Reactor System (CBR 90) comes with and without a stir plate and support stand. These assembly directions assume a stir plate and support stand are used.

1.1 Stir Plate and Support Stand:

1.1.1 Fix the aluminum rod into the back of the stir plate in the support clamp.

1.1.2 Mount the support ring in a clamp and attach to the support rod. Mount the ring low on the stand near the top of the stir plate.

1.1.3 Mount the 3-prong clamp onto the support rod with a clamp. Mount the 3-prong clamp high on the support rod.

1.2 Reactor Assembly:

1.2.1 Nutrient Supply Tubing: Attach tubing from your media reservoir to the glass flow break inlets.

1.2.2 Attach effluent tubing. Use minimum 1/4"(6 mm) ID and direct the effluent tubing to the drain or collection reservoir.

1.2.3 Insert the coupons into the coupon rods, flush with the inner rod surface.

1.2.4 Insert the rotor into the glass reactor vessel.

1.2.5 Mount the reactor lid onto the glass vessel while inserting the glass rod into the center of the blade rotor.

1.2.6 Insert the glass vessel bottom through the stand ring support. Adjust the location of the ring support to center the glass vessel in the middle of the stir plate.

1.2.7 Adjust the 3-prong support clamp to hold the influent tubing and glass flow breaks above the reactor vessel.

1.2.8 Insert rod coupon holders into the reactor vessel through the top access ports. Turn the coupon holders so the face of the coupons is perpendicular to the blade on the vane rotor (stainless steel alignment pin aligns with slot on the reactor lid), and push the coupon holders down into the ports.

7. CDC Biofilm Reactor Ancillary Equipment List

NOTE: BioSurface Technologies does not sell or supply the ancillary equipment described below. This or comparable equipment is required to complete the reactor system set-up. Pricing listed below may not be current. Please check with your local vendors for availability and current pricing.

There are many type of peristaltic pumps available and you may opt for alternative types and pump head configurations (multi-channel versus single channel, higher/lower rpm range, etc.). Depending on what you need to add to the reactor during operation, you may require more than 1 pump or a multi-head pump (media for growth and biofilm treatment chemical for some duration that may require an additional pump). The tubing you choose depends on the chemical compatibility, the gas permeability, wear resistance in peristaltic pumps, and pricing. You must choose the tubing that best fits your needs.

The pump, tubing, reservoirs, and accessories we commonly use is provided from Cole Parmer:

- **Masterflex L/S Precision Modular Drive w/ Remote I/O; 100 rpm**
  (P/N WU-07557-10 VAC CE certified; current list price: $1,183.00)
- **Masterflex L/S Easy-Load II Head for Precision Tubing, PPS/SS**
  (P/N EW-77200-60; current list price: $337.00)
- **Masterflex, C-Flex (50 A), L/S 16, 25 ft.**
  (P/N EW-06424-16; current list price: $42.75/pk)
- **Masterflex, C-Flex (50 A), L/S 18, 25 ft.**
  (P/N EW-06424-18; current list price: $62.25/pk)
- **Cole-Parmer Heavy-Duty PP Carboy w/ Shoulder Handle, 10 L**
  (P/N EW-62507-10; current list price: $60.50)
- **Filling/Venting ports for carboy lid-1/4" tubing**
  (P/N EW-06259-10; current list price: $25.00/pk)
- **Filling/Venting ports for carboy lid-1/2" tubing**
  (P/N EW-06259-00; current list price: $28.25/pk)

The size 16 tubing listed above is to fit the 3/16" barbed port fittings on the top of the CBR and connect to the media supply reservoir. C-Flex tubing is similar to silicone tubing but has a low gas- permeability compared to silicone. PharMed is also compatible for low gas -permeability. If gas-permeability isn't an issue, silicone tubing is acceptable. The size 18 tubing is for the effluent port on the reactor to the spent media collection vessel. The effluent port is a 3/8" barbed port and will accept any tubing of this diameter, again C-flex tubing low gas-permeability tubing is not required. You will also need a few lengths of 1/4" tubing and adapters to get from the carboy to the smaller diameter tubing, and as a siphon tube inside the carboy. If silicone tubing is acceptable (higher gas permeability), then size 25 tubing (ID of 3/16", less expensive; **EW-95802-09**; list price $56.25) can be used and pressed over the reservoir fittings, if other tubing type is required, then a size 17 tubing (ID 1/4" more expensive) is probably required (C-Flex: **EW-06424-72**, $63.25 list price). Adapters to get from the 3/16" or 1/4" ID to the 1/8" tubing are also required (Nylon 1/4" x 1/8" adapter: P/N **EW-30622-28**, pk of 10 list price $14.75).

Cole Parmer: 800-323-4340 (www.coleparmer.com)

8. RTD Temperature Probe
(for use with the digital stir/hot plate)

The CDC Biofilm Reactor with Stir/Hot Plate is provided with an RTD Temperature Sensor for temperature control of the Stir/Hot Plate. The standard fitting provided for the RTD Sensor is a Tee fitting that mounts on the vessel effluent port. The location of the temperature sensor in the effluent flow is critical to providing an accurate temperature measurement of the liquid media within the reactor. The RTD sensor tip should be placed in contact with the effluent flow as close to the reactor vessel as possible. At higher temperature operations, the sensor may need to be inserted into the reactor vessel to provide accurate temperature control. The RTD sensor may be inserted into the effluent port using the Tee fitting (inserted the length of the base of the Tee, with the effluent flow exiting to the waste collection vessel through the perpendicular port on the Tee fitting; make sure the fitting, sensor seal, and vessel port are liquid tight prior to operation).

ALTERNATE RTD SENSOR LOCATIONS

Through a Coupon Holder Rod Port: The RTD sensor may be mounted through one of the top ports (coupon holder rod ports) using a properly sized tapered hole stopper, bored to fit the temperature probe. This location allows direct contact of the reactor media to the temperature sensor and will provide the most accurate temperature control.

Center Glass Shaft Replacement with Sensor Well: The central glass shaft can be replaced with an RTD sensor well (p/n CBR 2218). This well consists of nylon tubing sealed at the bottom. The well allows insertion of the sensor into the reactor vessel, but does not directly contact the liquid. This location of the RTD Sensor allows accurate temperature control, but reduced temperature change response times.

9. RTD Temperature Probe Well Installation (for use with the digital stir/hot plate)

The central glass shaft can be replaced with an RTD sensor well (p/n CBR 2218). This well consists of rigid nylon tubing sealed at the bottom. The well allows insertion of the sensor into the reactor vessel, but does not directly contact the liquid. This location of the RTD sensor allows accurate temperature control, but reduced temperature change response times.

To replace the glass shaft with the temperature well, two 11/16" sockets (6-point, 18 mm) and socket drive are required. Place one of the sockets in a vise, pointing up. Insert the CDC lid with glass shaft into the immobilized socket, and align the hex nut on the bottom side of the lid into the hex socket. Place the other socket onto the hex nut on the top of the lid. Using the socket driver, turn the nut and lid counter-clockwise to loosen the bottom nut. Remove the bottom nut(If the top nut loosens before the bottom nut, place a flat bladed screw driver into the lid recess next to the compression fitting holding the glass rod and apply pressure to the screw driver to prevent the compression fitting from rotating, while turning the lid counter-clockwise. This rotation will loosen the compression nut on the bottom of the lid.). Press the bulkhead compression fitting out of the lid (the bulkhead compression fitting is not threaded to the lid). Insert the new bulkhead fitting with sensor well and compression gasket installed into the lid and install the bottom hex nut. Place the lid onto the socket held in the vise, and tighten the top hex nut using the socket and driver just until the top bulkhead gasket is compressed (remove the top socket, look down into the compression fitting recess. The compression gasket will start to bulge out when it is compressed. Over-compression will cause the gasket to bulge on one side significantly and un-seat the gasket from between the two mating surfaces).

The RTD sensor well should now be ready for use. The RTD sensor will slide into the well from the top of the reactor. The RTD sensor does not directly contact the liquid in the vessel, which reduces its response time. Allow extra time for the stir/hot plate to bring the temperature within the vessel to the desired set-point. Remove the sensor from the well prior to autoclaving the vessel.