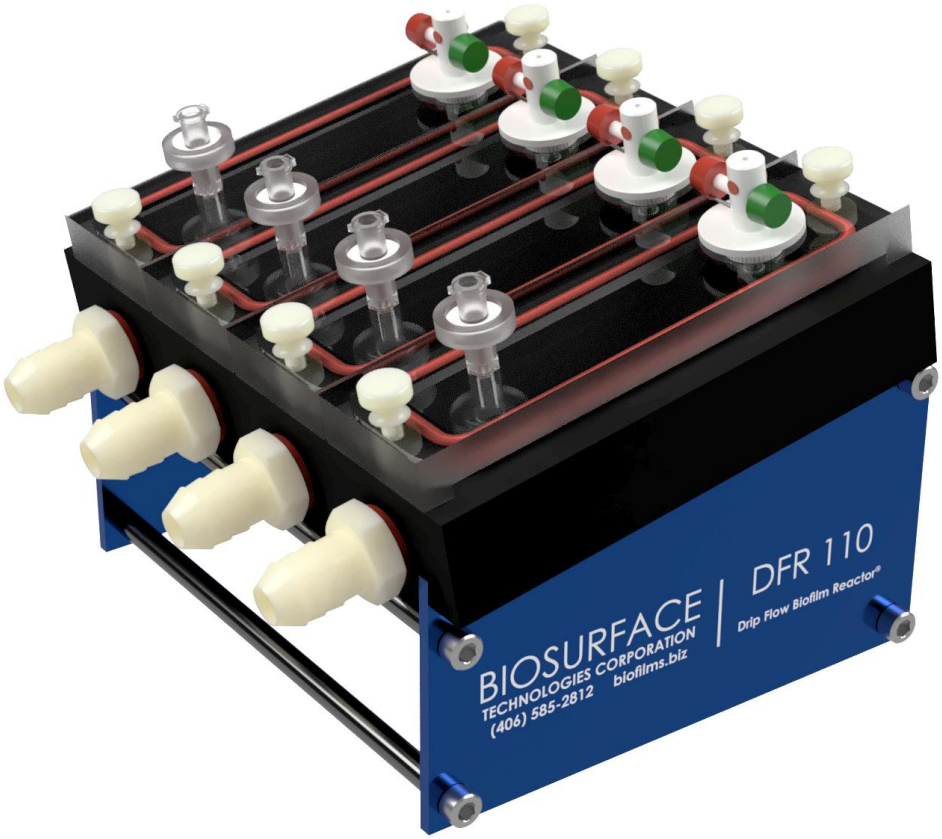
BioSurface Technologies Corporation

Drip Flow Biofilm Reactor® (DFR) Operator's Manual



Prepared with help from the Center for Biofilm Engineering, Standardized Biofilm Methods Laboratory for BioSurface Technologies Corporation

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1. Purpose

The Drip Flow Biofilm Reactor (DFR) Operator's Manual is intended to serve as a guide for researchers interested in growing a laboratory biofilm under low shear conditions at the air-liquid interface. It is the responsibility of the user to be familiar with basic microbiological concepts and techniques. Although a specific method is presented in this manual, the Drip Flow Biofilm Reactor (DFR) is suitable for modeling many different environments. Laboratory biofilms are engineered. The dynamics in the reactor determine the characteristics that describe a particular biofilm. No one biofilm, or biofilm reactor, is better than any other, although a particular reactor may be a more appropriate choice for modeling a certain environment. It is the responsibility of the operator to choose which reactor best suits their research needs.

2. Drip Flow Biofilm Reactor® Description

The DFR consists of a rectangular base (various materials available) held at a 10^{o} angle by an anodized aluminum stand, Figure 1. Four or six separate channels are bored into the base resulting in independent sampling opportunities for each run performed. Each channel has two small pegs to hold the $18.75cm^2$ (25 x 75 x 1mm) glass coupon in place, a shallow trough that mitigates blockage of the effluent port during sloughing events and aids in coupon removal, and an effluent port which allows the continuous flow media to exit. Each channel also has an alternant influent port that can be used for catheter studies. The covers contain rubber O-rings to form an airtight seal, bacterial air vent gas exchange ports, and a Mininert Valve used for the inlet. The Mininert Valve consists of a rubber septum, into which a needle is placed to deliver the media, and a ported bottom to allow for larger drops of media to form than is possible with the needle alone. The flow of media is the only acting shear force on the biofilm.

A. Material Selection

a. Polysulfone

Polysulfone is the original yellowish-clear plastic. It is autoclavable and resistant to acidic chemistries, but very fragile (crystalline plastic that easily cracks or crazes).

b. Polyethylene Terephthalate

Black Polyethylene Terephthalate (PET) has similar chemical resistance as Polysulfone but is much more resistant to physical and thermal stress (amorphous plastic without fracture planes). It is also a slightly less expensive option. PET is an opaque autoclavable thermoplastic.

Both plastic options will eventually decay from repeated autoclave cycles.

c. Anodized Aluminum

Anodized Aluminum is very resistant to thermal and physical stress. The anodized coating is very thin and can be scratched easily. Once the anodized coating is removed, the underlying aluminum metal is very reactive in salt-containing solutions (growth media). The anodized coating will decay after repeated exposure to acidic conditions. The coating can be reapplied by reanodizing.

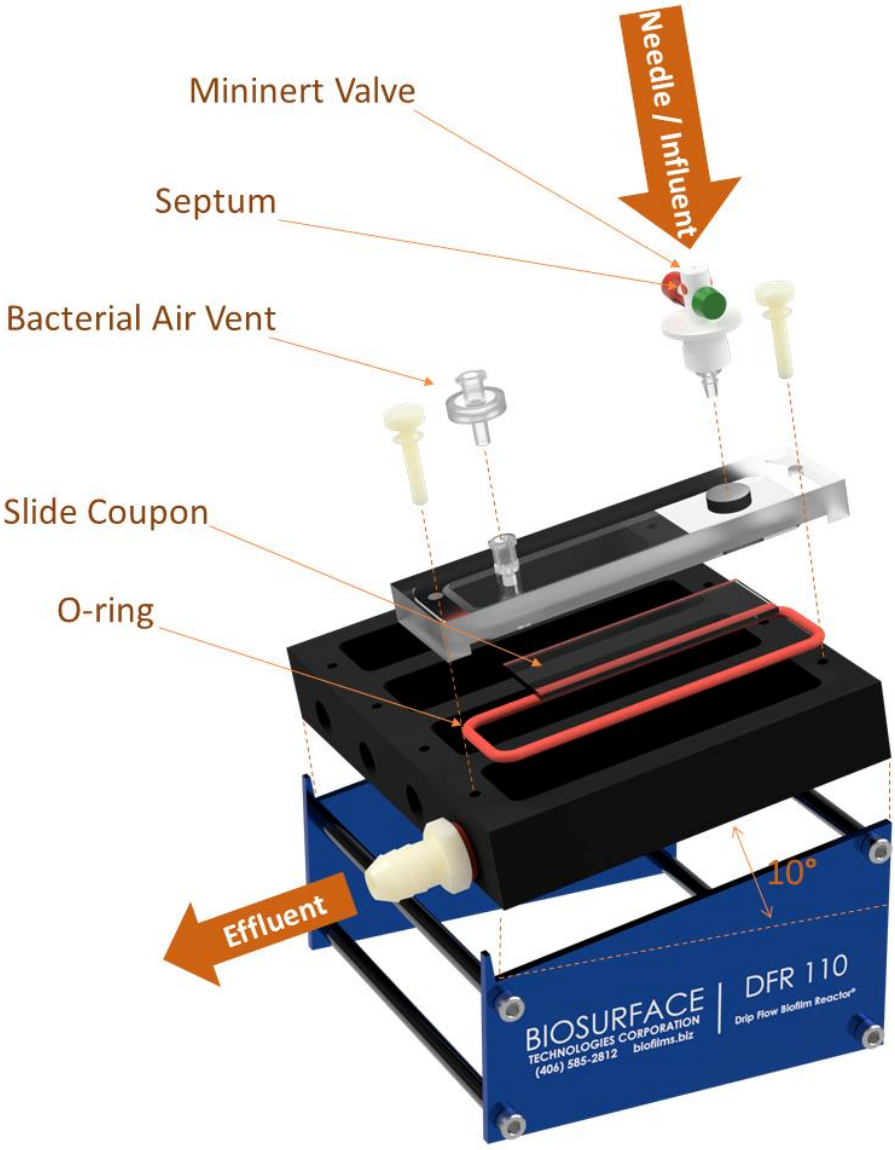


Figure 1. Drip Flow Biofilm Reactor Schematic.

3. Drip Flow Biofilm Reactor® Standard Operating Procedure

The DFR 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) describes one method for growing a repeatable *Pseudomonas aeruginosa* biofilm under low shear at room temperature. Operators are encouraged to modify the protocol to model the most appropriate biofilm for their research needs, although changing the protocol and/or reactor configuration will require the operator to retest the repeatability of the new method. 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 biosafety recommendations for each individual species. 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 microorganisms living in a self-organized, cooperative community attached to surfaces, interfaces, or each other, embedded in a matrix of extracellular polymeric substances of microbial origin, while exhibiting an altered phenotype with respect to growth rate and gene transcription. Biofilms may be comprised of bacteria, fungi, algae, protozoa, viruses, or infinite combinations of these microorganisms. The qualitative characteristics of a biofilm (including, but not limited to, population density, taxonomic diversity, thickness, chemical gradients, chemical composition, consistency, and other materials in the matrix that are not produced by the biofilm microorganisms) are controlled by the physicochemical environment in which it exists. In this method, a laboratory biofilm is established in batch mode for six hours and is then grown under low shear in continuous flow conditions for 48 hours. Biofilm accumulation is quantified by harvesting the biofilm from coupons of a known surface area, disaggregating the cell clumps and performing viable plate counts.

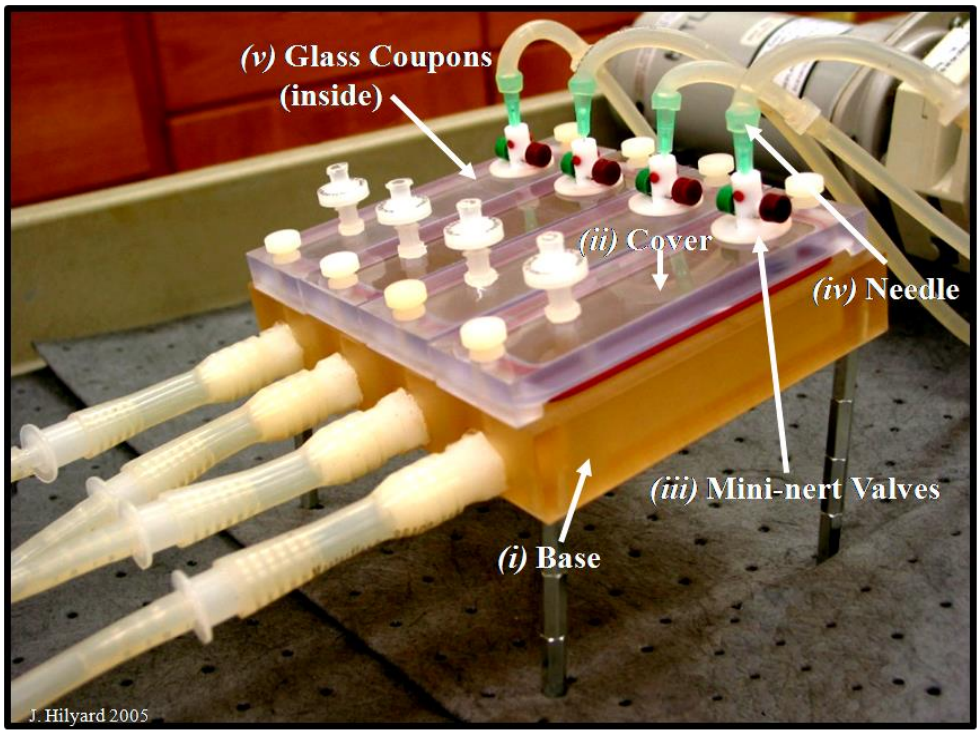


Figure 2. Reactor Components. [Roman numerals refer to description in section *B(a)*]

B. Supplies

(a) Reactor components

i) Base – polysulfone, PET, or anodized aluminum base with 4 or 6 channels and effluent ports (one at the end of each channel). The reactor sits on an aluminum stand, providing a 10^{o} angle of operation (Figure 2 shows an older model).

ii) Cover – 4 or 6 polycarbonate covers, each with 2 threaded holes for nylon screws to secure to reactor base. Two ports, one for influent media line attachment and another for bacterial air vent attachment (used to allow for sterile air and gas exchange). O-rings fitted underneath to seal cover to base during operation (Figure 2).

iii) Mininert Valves – fit into each cover as influent ports to allow inoculation and media line attachment (Figure 2).

iv) Needle – 1 inch, 14 to 22 gauge (not provided); fit into Mininert Valve (Figure 2).

v) Glass coupons – 4 or 6 new rectangular glass microscope slides with a surface area of 18.75 cm^2 (25 x 75 x 1 mm) (Figure 2).

NOTE 1: Coupons may be fashioned from other similarly shaped/sized materials, however, the test method must be re-evaluated and standardized accordingly.

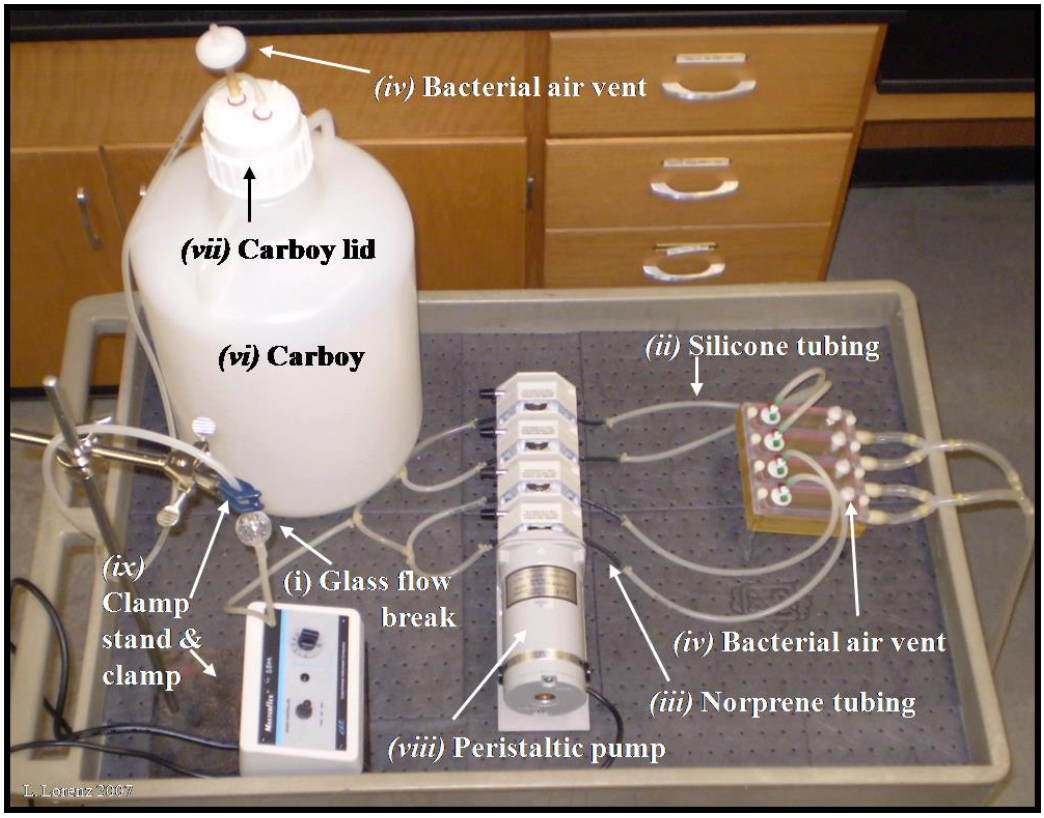


Figure 3. Assembled Apparatus Components
Roman numerals refer to description in section *B(b)*.

(b) Assembled apparatus components

i) Glass flow break – any that will connect with tubing of ID 3.1 mm and withstands sterilization (Figure 3). Used to prevent back-contamination into the media carboy.

ii) Silicone tubing – two sizes of tubing: one with ID 3.1 mm (size 16) and the other with ID 7.9 mm (size 18). Both tubing sizes must withstand sterilization (Figure 3).

iii) Norprene tubing – with ID of 3.1 mm (size 16), capable of withstanding sterilization. This section is used for running through the pump head (Figure 3).

NOTE 2: Other tubing materials and sizes may be used provided that the test method is carefully re-evaluated. Ensure that ID changes are taken into consideration for flow rate calculations if Norprene tubing substitutions are made.

iv) Bacterial air vent – autoclavable 0.2 micrometer pore size, spliced into tubing on waste carboy and nutrient carboy. Attach small air vent to port on each reactor channel cover (Figure 3).

v) Teflon thread seal tape – wrapped around effluent ports, if needed, to prevent leakage from effluent port connectors.
vi) Carboys – two 20 L autoclavable carboys, used for waste and nutrients (Figure 3).
vii) Carboy lids – one carboy lid with at least 2 barbed fittings to accommodate tubing ID 3.1 mm (one for nutrient line and one for bacterial air vent). One carboy lid with at least two 1 cm holes bored in the same fashion (one for effluent waste and one for bacterial air vent).

NOTE 3: Carboy lids can be purchased pre-drilled and/or fitted or can be purchased for drilling/fitting in-house by the user.

viii) Peristaltic pump – with four pump heads. Capable of holding tubing with ID 3.1 mm and OD 3.2 mm and calibrated to operate at a flow rate of 200 mL per hour (Figure 3).
ix) Clamp stand and clamp – to hold glass flow break and media inlet tubing in a vertical position. Clamp with 0.5 cm minimum grip size.

(c) Media and culture preparation supplies
i) Analytical balance – sensitive to 0.01 g +/- 0.02 g.
ii) Sterilizers – any steam sterilizer capable of producing the conditions of sterilization.
iii) Environmental shaker – capable of maintaining temperature of 35^{o}C +/- 2^{o}C.

(d) Sampling supplies
i) Pipettes – continuously adjustable pipette with volume capability of 1 mL +/- 8 µL (for dilutions and rinsing). Automatic or manual pipette with dispensing volume of 0.01 mL +/- 0.8 µL for drop plating.
ii) Homogenizer – any capable of mixing at 20,500 +/- 5,000 rpm.
iii) Homogenizer probe – any capable of mixing at 20,500 +/- 5,000 rpm in a 50 mL volume and able to withstand autoclaving or other means of sterilization.
iv) Vortex – any vortex mixer that will ensure proper agitation and mixing of culture tubes.
v) Teflon, metal or rubber spatulas – sterile, for scraping biofilm from coupon surface.
vi) Bunsen or alcohol burner – used to flame sterilize inoculating loop and other instruments. 95% ethanol used to flame sterilize hemostats or forceps.
vii) Culture tubes and culture tube closures – any with a volume capability of 10 mL and a minimum diameter of 16 mm. Recommended size is 16 x 125 mm borosilicate glass with threaded opening.
viii) Petri dish – 100 x 15 mm, plastic, sterile and empty for transporting coupon holders from reactor to workstation.
ix) Stainless steel hemostat clamp or forceps – for aseptic handling of coupons.
x) Glass beakers – sterile, any with a volume capacity of 100 mL containing 45 mL sterile dilution water.
xi) Conical-bottom sterile disposable plastic centrifuge tubes – with a volume capacity of 50 mL (containing 45 mL sterile dilution water).

C. Media and Reagents

NOTE 4: All reference to water as diluent or reagent means distilled water or water of equal purity.

(a) Inoculum culture media – 100 mL of 3,000 mg Tryptic Soy Broth (TSB)/L. Sterilize for 20 minutes on liquid cycle.

(b) Batch culture media - 100 mL of 3,000 mg TSB/L. Sterilize for 20 minutes on liquid cycle

(c) Continuous flow media – Prepare continuous flow nutrient broth by dissolving bacterial liquid growth medium in 20 liters sterile reagent grade water. Dissolve and sterilize the broth in a smaller volume to prevent caramelization. Aseptically pour the concentrated broth into the carboy of sterile water to make a total of 20 liters of growth media at a concentration equal to 270 mg TSB/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 conical vials and beakers with 45 mL of sterile dilution water. (Buffered dilution water preparation – Method 9050 C.1*a*)[1]

(e) Bacterial plating medium – R2A agar prepared according to manufacturer's recommendations.

(f) 95% ethanol – For dipping hemostat prior to flame sterilization.

(g) 70% ethanol – For rinsing homogenizer probe and general bench top clean up.

D. General Instructions

(a) Assembling the reactor for autoclaving

i) Use new coupons for every experiment.

NOTE 5: Coupons can be used once and discarded or used repeatedly with proper cleaning and sterilization between each use. Check each coupon for scratching, chipping, other damage or accumulated debris before each use by screening under a dissecting microscope at a magnification of at least 20X. Discard those with visible damage to surface topography.

ii) Insert a coupon into each reactor channel, positioning one end of the slide directly under the influent media port and allowing the opposite end of the slide to rest on the pegs near the effluent port of the channel.

iii) Place channel covers onto base and loosely screw in nylon screws.

NOTE 6: Nylon screws should be loosened when sterilizing the reactor to prevent difficult removal after autoclaving.

iv) Attach the bacterial air vent.

v) Splice the glass flow break into the area of the media tubing line that will be near the carboy top when attached.

[1] Eaton, A.D., L.S. Clesceri, A.E. Greenberg. (Eds.) 1995. Standard Methods for the Examination of Water and Waste Water, 19th Edition. American Public Health Association, American Water Works Association, Water Environment Federation. Washington D.C.

vi) Configure the media tubing so that four individual lines result. The four lines will each be fed through a pump head then attached to a needle, which are inserted through the Mininert Valves feeding the channels as shown in Figures 2 and 3.
vii) To sterilize the reactor, remove the adjustable legs from the reactor base. Wrap all exposed tubing ends and openings with aluminum foil, clamp all effluent tubing and place assembled reactor into an autoclave tray. Cover entire tray with aluminum foil.

(b) Sterilizing the reactor – Sterilize the reactor system for 20 minutes on liquid cycle. Remove immediately after cycle is finished to prevent cracking of the reactor base.

(c) Setting the pump flow rates

NOTE 7: Pump flow rates should be set prior to performing the experiment. The same ID tubing should be used to set the flow rate as will be used in the experiment. It is not necessary to use sterile tubing when calibrating the pump.

i) To set flow rate, turn on pump and let warm up for 1-2 hr.
ii) After warming up, turn off pump and carefully assemble tubing through the pump heads. Seat firmly to prevent creeping.
iii) Place one end of the tubing into a beaker of water and each of the four other ends into separate graduated cylinders.
iv) Turn on the pump to the approximate desired flow setting (or start with 1 on the dial).
v) Let the water flow through the tubing and start the timer when drops begin to fall into the graduated cylinders. Time for 10 min.
vi) Divide volume in each cylinder by 10 min to determine flow rate in mL/min for the corresponding channel. Adjust the dial accordingly until the desired flow rate of 0.8 mL/min/channel is achieved.

(d) Bacterial culture and inoculum preparation

i) Streak for isolation, a frozen stock culture of *Pseudomonas aeruginosa* (ATCC 700888), on an R2A agar plate.
ii) Allow the plate to incubate at 35^{o}C for 17-24 hours.
iii) Obtain an isolated colony from the streak plate described above and stir into inoculum culture media (*C.a*). Incubate at 35 +/- 2^{o}C while shaking for 18-24 hours.

NOTE 8: 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.

(e) Batch phase

i) Place the cooled reactor in a flat, level position on the bench top.
ii) Clamp flow break in upright position; leave other tubing clamped and foiled.
iii) Attach effluent tubing to waste carboy.
iv) Aseptically add 15 mL of 3,000 mg TSB/L (*C.b*) to each channel using a sterile serological pipette. Pipette 1 mL of inoculum (*D.d.iii*) into each channel and tighten the cover securely (hand tight) with nylon screws.

(f) Adjusting the reactor angle

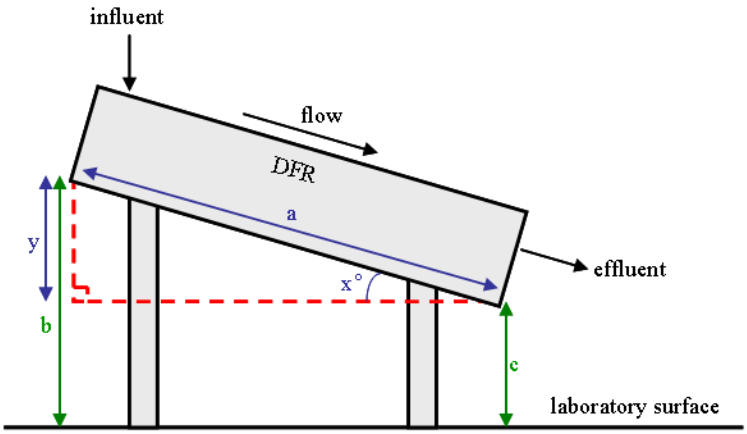


Figure 4. Side view of the DFR used for calculating the reactor angle.

i) Measure a, the length of the reactor base, in cm.
ii) Determine required angle (x). (This protocol requires a 10° angle).
iii) Calculate y using the following equation:

$$y = a[\sin(x)]$$

a=measured length of reactor base in cm
x=required angle
y=difference between length of b and length of c in cm.

iv) Decide upon the lengths of b and c to obtain the required difference (y). Use the following equation:

$$y = b - c$$

b = measured length (cm) from bottom corner of influent end of reactor base to laboratory surface
c = measured length (cm) from bottom corner of effluent end of reactor base to laboratory surface
y = difference between length of b and length of c in cm.

NOTE 9: Determine the necessary lengths of b and c before starting an experiment. To do this, first calculate y using $y = a[\sin(x)]$. Set length c by tightening the legs near the effluent port until hand tight. Using the equation $y = b - c$, determine length b and adjust legs near influent port accordingly. (Note: Only possible when using an older model, with adjustable legs)

(g) Continuous flow phase

i) After 6 hours of batch conditions, unclamp the effluent tubing and attach the legs to the DFR.
ii) Adjust the legs in the reactor base until the required lengths (b and c) are achieved so that it slopes downward 10° (*D.f*).
iii) Aseptically connect the influent nutrient tubing line to the carboy containing the continuous flow nutrient broth. Feed each of the four lines through a pump head and connect a sterile needle on the end of each tube.
iv) Aseptically insert the sterile needles through the Mininert Valves in the channel covers.

NOTE 10: Use standard precautions when using needles.

v) Turn on the pump and allow media to slowly drip onto the bacterial cells attached to the coupon.

NOTE 11: The media should flow downward from the influent port to the effluent port. Periodically check the reactor for proper drainage and check the effluent tubing for leaks.

vi) The reactor is operated in Continuous Flow mode for 48 hours.

E. Sampling

(a) Sampling the biofilm

i) Prepare sampling materials: vortex, homogenizer, sterile beakers, sterile centrifuge tubes, culture tubes, pipettes, empty sterile Petri dish, sterile spatulas, and flame sterilized stainless steel hemostat or forceps.

NOTE 12: Be sure to wear proper personal protective equipment including a lab coat, safety glasses, and gloves.

ii) Carefully loosen channel cover screws and remove the channel cover. Aseptically remove one of the coupons by gently lifting the coupon out of the channel with sterile forceps. Hold coupon over a sterile Petri dish while moving it to the sampling area.
iii) Be careful not to disturb the attached biofilm while holding the coupon. Sterilize the hemostat between each coupon sampling.
iv) Rinse the slide to remove planktonic cells. Hold slide and centrifuge tube at a 45^{o} angle. Uncap tube and gently immerse slide with a fluid motion until slide is completely covered. Immediately reverse motion to remove the slide.
v) Scrape biofilm-covered coupon surface in a downward direction for approximately 15 seconds, using the flat end of a sterile spatula or scraper, into the beaker containing 45 mL of sterile dilution buffer. Rinse the spatula or scraper by stirring it in the beaker. Repeat the scraping and rinsing process 3-4 times, ensuring full coverage of the coupon surface.
vi) The coupon is rinsed by holding it at a 60^{o} angle over the sterile beaker and pipetting 1 mL of sterile dilution water over the surface of the coupon. Repeat for a total of 5 rinses. The final volume in the beaker is 50 mL.

(b) Disaggregation – The scraped biofilm sample is homogenized in the beaker at 20,500 +/- 5,000 rpm for 30 seconds. Between every sample, clean the homogenizing probe as follows: homogenize a dilution blank for 30 seconds at the same rpm, homogenize a tube containing 70% ethanol for 15 seconds, remove the probe and let it sit in the ethanol tube for 1 minute, shake any remaining ethanol off the probe, reattach it, homogenize a dilution blank for 30 seconds and finally homogenize a second dilution blank. Now the probe is ready to homogenize the next sample tube. Discard 70% ethanol at end of sampling.

NOTE 13: Homogenizing the sample disaggregates the biofilm clumps to form a homogenous cell suspension. Improper disaggregation will result in an underestimation of the viable cells present in the sample.

(c) Serial dilution – Serially dilute the sample 1:10 in buffered dilution water using sterile culture tubes.

(d) Viable plate counts

i) Plate each dilution in duplicate for colony growth using an accepted plating technique such as spread, spiral, or drop plating.
ii) Incubate the plates for 17-24 hours at 35 +/- 2°C.
iii) Count the appropriate number of colonies according to the plating method used.

F. Calculations

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

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

b) Calculate the overall biofilm accumulation by taking the mean of the log densities calculated in section (*E.a*).

G. Repeatability

For the DFR SOP, the estimated repeatability standard deviation is 0.28 of which 65% is attributable to within-experiment sources and 35% is attributable to between-experiment sources. 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 $[(0.05065/n) + 0.02711]^{1/2}$. For example, if the protocol specifies n=4 coupons, the repeatability standard deviation is 0.20 of which 32% is attributable to within-experiment variation and 68% is attributable to between-experiment variation.

H. Troubleshooting

(a) Tubing

NOTE 14: Most problems that arise with this reactor system are due to issues with compromised tubing, therefore, it is necessary to replace all the tubing periodically.

i) Ensure tubing and plastic connectors are autoclavable prior to use.
ii) To prevent leakage, ensure there is a tight connection between the tubing and the luer lock connectors (where the needles will be attached) – tighten if necessary, using plastic ties.
iii) Check to ensure that there are no obstructions of flow in any tubing lines.
iv) Be sure to gently seat tubing in the pump heads to avoid clamping and puncture of the tubing lines.
v) Blockage of the effluent tubing may occur if the tubing is too narrow when fungal or very thick biofilms are being grown. This will result in backflow into the channels and leakage.

(b) Reactor Base

NOTE 15: It is important to follow proper autoclaving procedures to prevent premature deterioration of the reactor system. The reactor should be autoclaved for 20 minutes on liquid cycle, with the cover screws loosened and the legs removed.

i) Check entire base for cracks, paying close attention to effluent connectors.
ii) Verify that channels are clean and cleared of anything that could plug effluent flow.

(c) Miscellaneous

i) During batch mode, ensure that the reactor is sitting as level as possible and that all slides are completely covered with inoculum.
ii) Be sure to remove effluent clamps before beginning continuous flow.
iii) Change air vents periodically or if they become wet (as this will prevent gas transfer).
iv) Replace membrane septa in the Mininert Valves, as necessary.

I. Acknowledgement

The development and standardization of the Drip Flow Biofilm Reactor® was funded by a grant from The Montana Board of Research and Commercialization Technology.

4. Drip Flow 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. Please check with your local vendors for availability and current pricing. Equipment listed is not recommended equipment, but an aid to help you identify compatible equipment.*

Pump: There are many types 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).

- **L/S Variable Speed Modular Drive Pump system (1-100 RPM)**
  (Cole Parmer P/N EW-07557-10; 90-230 VAC CE certified)
- **Ismatec Minicartridge Pump Heads for Masterflex L/S Drives**
  (Cole Parmer P/N EW-07623-10)
    - Has eight channels. One channel per reactor channel needed to operate. Will accommodate the 4 separate flow channels on each of two DFR-4 reactors or one DFR-6 reactor.

Tubing and Fittings: The tubing you choose depends on the chemical compatibility, gas permeability, wear resistance in peristaltic pumps, and pricing. You must choose the tubing that best fits your needs. C-Flex tubing (listed below) is similar to silicone tubing but has a low gas-permeability compared to silicone. If gas-permeability is not an issue, standard silicone tubing is acceptable.

- **Masterflex, C-Flex (50 A), L/S 16, 25 ft.** (Cole Parmer P/N EW-06424-16)
    - To connect to the media supply reservoir (including through pump-head).
- **Male Luer Lock x 1/8" barb –Polypropylene** (Cole Parmer P/N WU-30800-24)
    - Four are included with standard reactor system.
- **Masterflex, C-Flex (50 A), ¼" ID x 7/16" OD, 25 ft**
  (Cole Parmer P/N EW-06424-72)
    - A few lengths and adapters to get from the carboy to the smaller diameter tubing, and as a siphon tube inside the carboy.
- **Nylon 1/4" x 1/8" adapter** (Cole Parmer P/N EW-30622-28)
    - Needed to get from the 3/16" or 1/4" ID to the 1/8" tubing.
- **Size I/P 82 tubing 25' C-Flex** (Cole Parmer P/N WU-06424-82)
    - For the effluent port on the reactor to the spent media collection vessel. The effluent port is a 1/2" barbed port and will accept any tubing of this diameter.

Carboy: The carboy should be selected based on experiment needs and may be larger or smaller than what is suggested below. Ported lids can be purchased from suppliers, but standard lids are easily converted to ported lids using the following fittings or similar.

- **Cole-Parmer Heavy-Duty PP Carboy with Shoulder Handle, 10 L**
  (Cole Parmer P/N EW-62507-10)
- **Filling / Venting ports for carboy lid - 1/4" tubing**
  (Cole Parmer P/N EW-06259-10)
    - Each reservoir vessel will require this port for the bacterial air vent.
- **Filling / Venting ports for carboy lid - 1/2" tubing**
  (Cole Parmer P/N EW-06259-00)
- **Bacterial vent for media and waste reservoirs**
  Any 0.22-0.45 um filter will work; can use pre-sterilized filters and mount on the vessels immediately after autoclaving.

- **PTFE Nonsterile Vent Filters for carboy venting-0.45 um; PP Housing**
  (Cole Parmer P/N: WU-02915-30)

Syringe Needle:

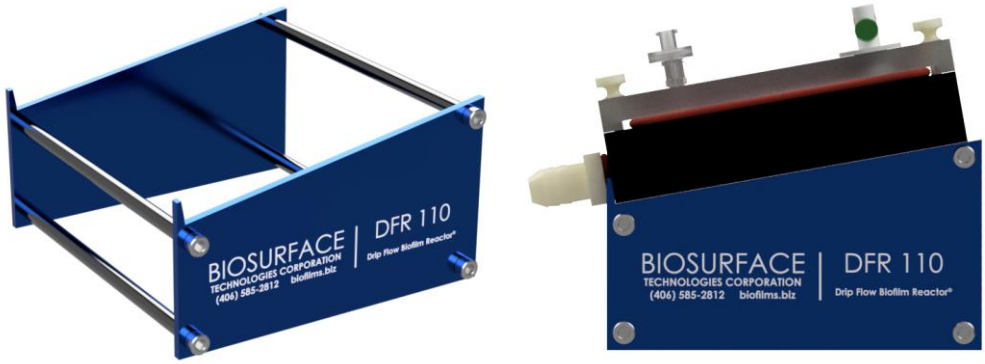
- **Disposable Syringe Needle with female luer connector**
  (Fisher Scientific P/N: 14-826-5A; BD PrecisionGlide Needles, 22 gauge, 1.5-inch length)
    - The luer / barb adapters (provided with DFR 110) will allow connection to disposable syringe needles with female luer connectors to the size 16 tubing. The disposable luer needles are used as influent lines through the Mininert Septa Ports. Syringe needles in the 18-24 gauge are compatible.

Ancillary Equipment Suppliers:

Cole Parmer: 800-323-4340 (www.coleparmer.com)
Fisher Scientific: 1 800-766-7000 (www.fishersci.com)

5. Drip Flow Biofilm Reactor® Base Support Assembly

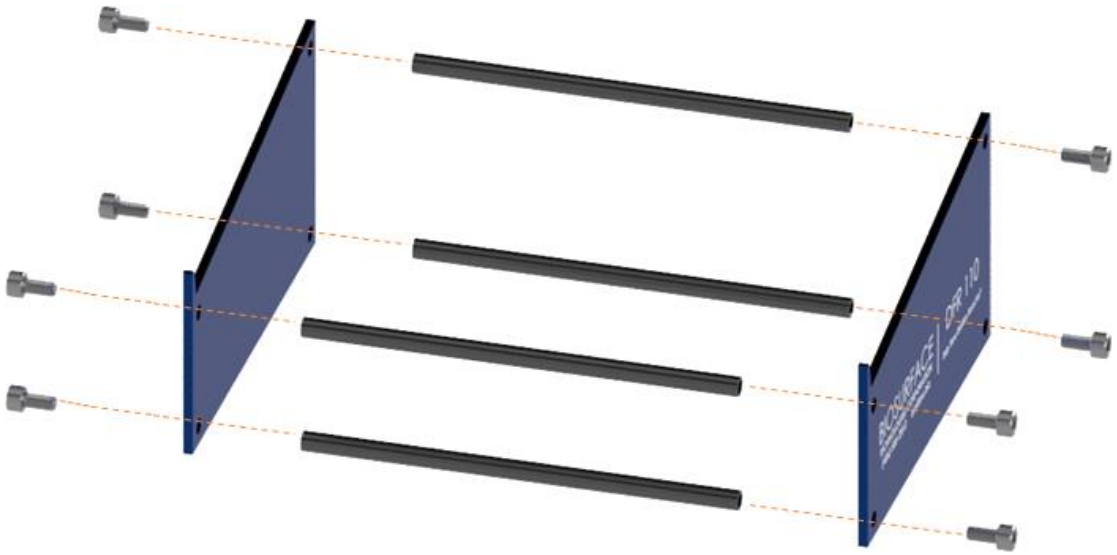
The Drip Flow Biofilm Reactor Base Support consists of blue anodized aluminum plates separated by 4 black anodized aluminum rods. The top angle of the plates is designed to provide the proper fluid flow through the reactor (10 degrees from horizontal).



To assemble the base support:

1) Insert the screws through a side plate. Thread the screws into each of the four rods so that the first side is secured to the rods.
2) Insert the remaining four screws into the second side plate, and thread these screws into the opposite end of the support rods.

NOTE: These screws can be hand tightened, or an Allen wrench can be used.



DFR1114-BS Assembly

6. Drip Flow Biofilm Reactor® Autoclaving

The Drip Flow Biofilm Reactor is made of polysulfone thermoplastic, black PET (Polyethylene Terephthalate) plastic, or anodized aluminum. These materials are fully compatible with autoclave temperatures up to 121°C. However, extended autoclaving times (greater than 20 minutes), numerous autoclave cycles, or higher temperatures will shorten the working life of the reactor, and is not covered under warranty. BioSurface Technologies Corporation recommends slow exhaust cycles and immediate removal from the autoclave at the end of the cycle. When autoclaving, loosen the threaded thumb screws that hold the lids in place to relieve stress on these areas of the reactor. Additionally, BioSurface Technologies Corporation does not recommend autoclaving with threaded stand-off legs inserted, as this may cause cracking or crazing around the threaded recesses. In addition, we recommend arranging the tubing connected to the reactor in a manner that it does not put stress on the effluent ports (align the tubing with the port and minimize side-ways stress or strain on the port fitting) while autoclaving.

Polysulfone material is especially susceptible to stress at the fittings during autoclaving and can result in cracking around these connections. The polysulfone material is fragile and may fracture or crack if mishandled. Treat the reactor as if it is made from glass and minimize thermal stress to the reactor (do not place the hot reactor from the autoclave onto a cold countertop).

7. Drip Flow Biofilm Reactor® Standard Methods

A. ASTM (www.astm.org)

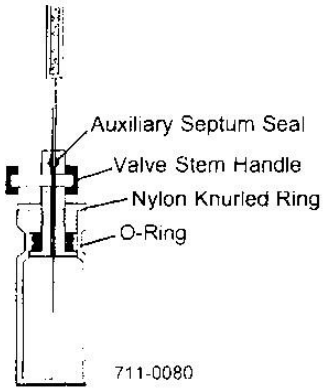
a. ASTM E2647

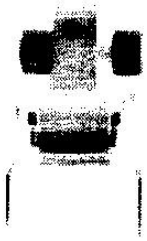
Standard Test Method for Quantification of a *Pseudomonas aeruginosa* Biofilm Grown Using a Drip Flow Biofilm Reactor with Low Shear and Continuous Flow

8. Operating Instructions for Mininert Valves

Operating Instructions for Mininert® Valves

(for serum bottles)

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To Install Valve:

1. Loosen the movable knurled ring.
2. Keep the bottle neck dry. If the bottle is filled with liquid, open the valve and insert a needle through the septum to release compression.
3. Insert the valve into the bottle.
4. Tighten the ring snugly (about two turns). **Do not overtighten** – it is okay if the valve rotates in the bottle neck.

To Remove Valve:

1. Unscrew the knurled ring.
2. Pull the valve up, twisting slightly.

To Open Valve:

1. Press the green button against the valve body.
2. The ports will align, permitting needle insertion for sampling. The auxiliary septum seal (if in good condition) will prevent sample loss or exposure.

To Close Valve:

1. Withdraw the needle and press the red button against the valve body.

To Replace the Auxiliary Septum:

1. Valve must be in closed position.
2. Push the old septum out of the valve using a septum inserter (Cat. No. 33311) or a smooth, rounded metal rod.
3. Gently press a new septum (Cat. No. 33310-U) into the valve and **work it in carefully** using a flat surface or a rod. Avoid excessive force that may distort the septum.

WARNING!
Pressurization of glass vessels can be dangerous.
Use glass vessels as pressurized containers,
with or without Mininert valves, at your own risk.

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SUPELCO
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