COMPARITIVE STUDY OF THE EFFECT OF SINGLE WALLED CARBON NANOTUBES ON *Escherichia coli* IN CULTURES AND BIOFILMS

A write-up on the proposed Study

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Study Overview:

Recent study by researchers at Yale University (1) has confirmed the toxicity of single walled carbon nano tubes (SWCNT) on the coliform bacteria *Escherichia coli*.

This study aims at studying the effect of single walled carbon nano tubes on the gram negative bacterial strain Escherichia coli k-12 both on cells in suspended culture, and biofilm associated cells. A CDC biofilm reactor is to be used.

Project Outline:

The study is divided into sub stages to help in planning the timeline for each stage:

I. Culture E. coli and develop cell count method:

Strains of E. coli K-12 and the protocol for E. coli culture will be obtained from doctoral student Yu Yong. The strain will then be aseptically batch cultured (appendix 1) in a flask, based on the protocol and will be left overnight for the bacteria to grow. Next day 1mL of the culture will be serially diluted to enable plate count of cells (i.e to obtain a final dilution of 25-250 cells per plate). The final dilution will then be filtered and the filter is placed on a petri-plate filled with media (agar). The Petri plate will then be incubated at around 32 °C for 24 hours. The colonies (each representing a cell in the dilution filtered) on each plate are then counted by placing it under appropriate magnification and lighting.

II. Experimental Setup with the biofilm reactor:

The CDC biofilm reactor would be used to grow the E. coli biofilms under high shear and continuous flow conditions. It consists of a 1 L beaker with 8 rods which have 3 coupons each. These coupons act as substrate for biofilm growth.

First, the bacterial culture (diluted) would be pumped through the reactor overnight to get the coupons coated with the bacterial strains. This could be recycled back to the beaker with culture. Then the culture media will be pumped through the bioreactor using a peristaltic pump. The reactors outlet is at 400mL mark and will result in a volume of approximately 350mL during operation. Thus it is to be noted due to pumping of the media and bacterial strains through the reactor there would be some amount of suspended culture growing in the reactor. Thus this setup will help us in studying both the cells in suspension and those associated with biofilm. All other growth conditions would be maintained favorable and it is assumed that the microbes are not under stress.

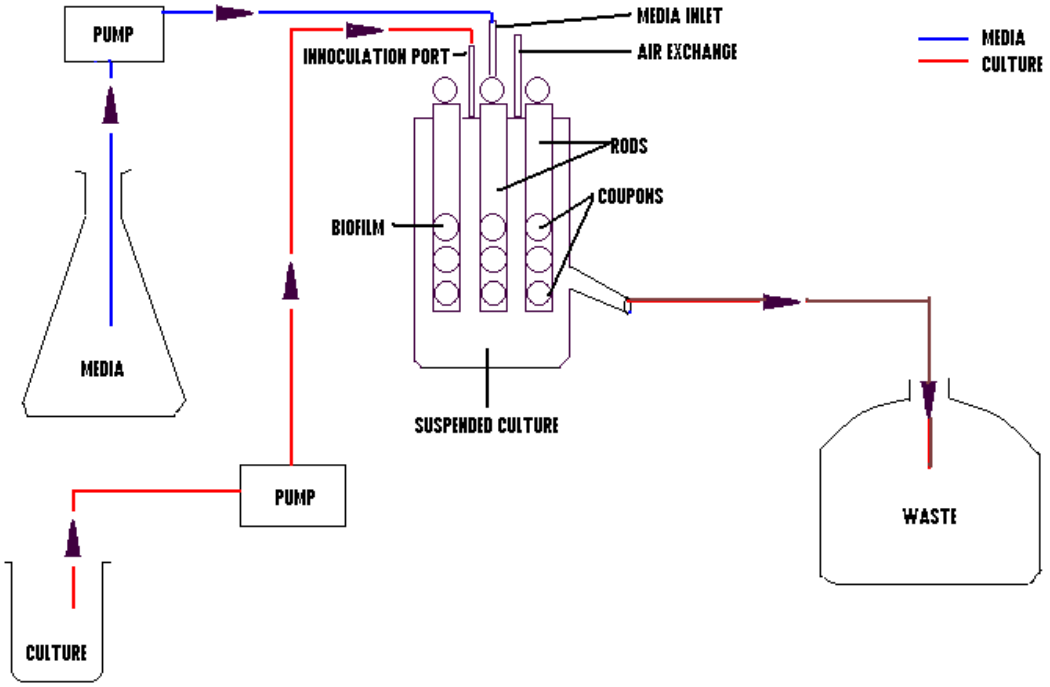

Figure1: CDC biofilm reactor setup for growing E. coli cells in suspension and in biofilm

III. Exposure of cells in biofilm and in suspension to SWCNT:

After allowing sufficient time for the biofilm to grow, one of the rods along with the three coupons on them would be removed from the biofilm reactor to do a plate count of the cells in the biofilm. The cells will be scrapped on to a Petri plate and then placed under sufficient magnification and light to do a plate count test. At the same time, a portion of the cells in the suspended culture in the biofilm reactor will be removed to do a plate count of the cells in suspension.

Following the count of cells in suspension and in biofilm at favorable conditions, the single walled carbon nano tube (SWCNT) solution would be pumped through the inoculation port. All other conditions would be kept favorable. One rod will be removed every day after exposure to SWCNT solution and to perform a plate count of cells on the coupons. Since the SWCNT exposure is said to kill the cells immediately the cell count after exposure will be made for 1-3 days.

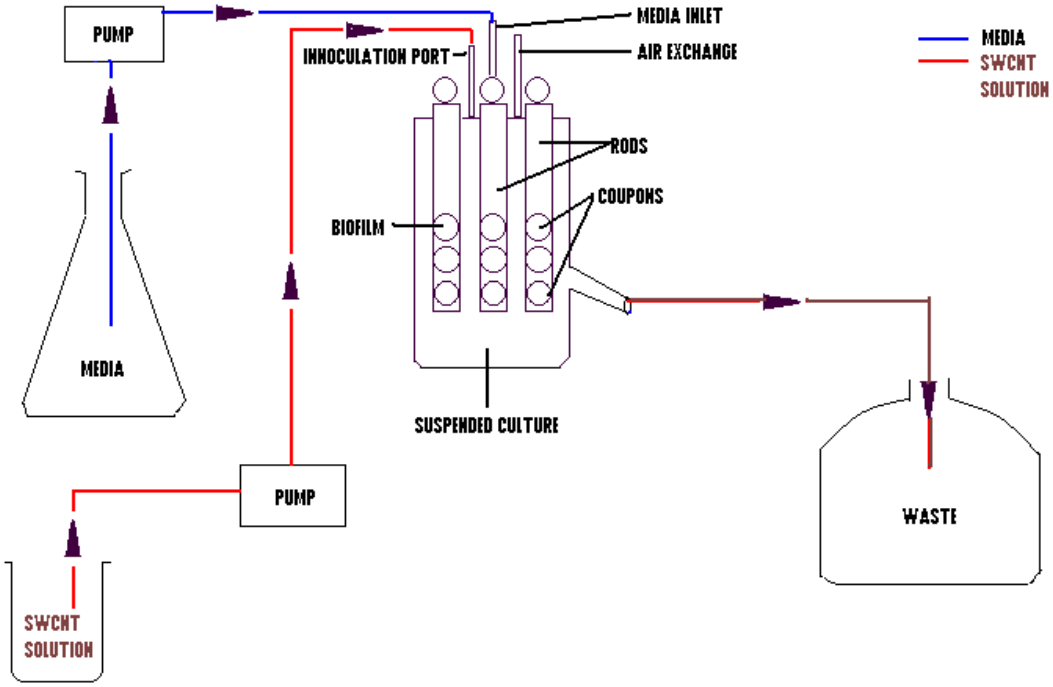

Figure 2: CDC biofilm reactor setup with cells in biofilm and suspension exposed to SWCNT solution

The cell counts are then plotted on a graph of Cells Vs time before and after exposure to SWCNT and a percentage reduction in total number of cells in biofilm and suspension is calculated.

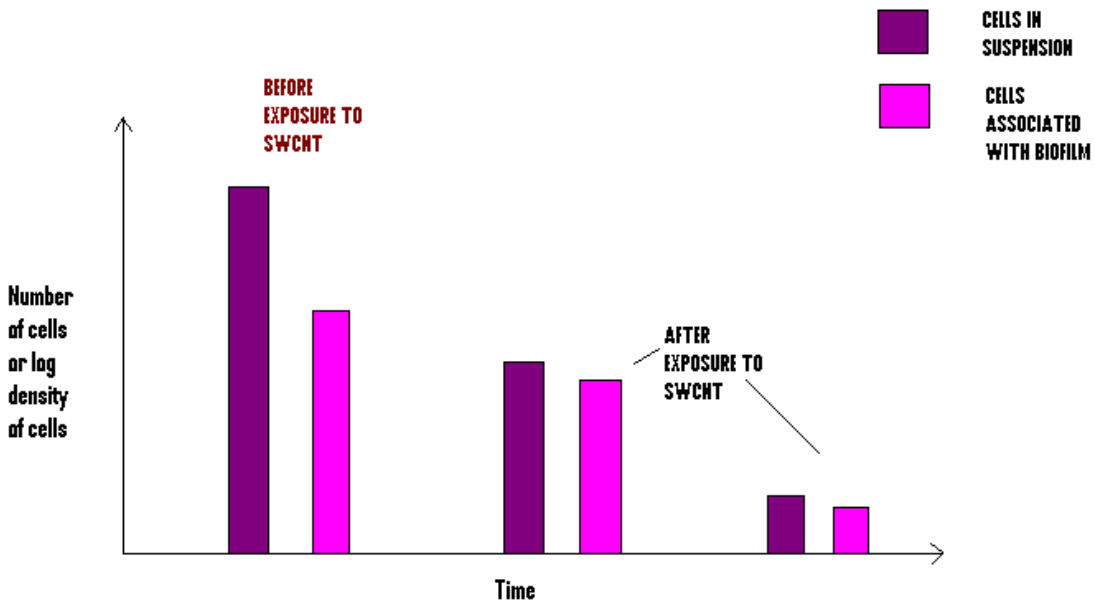

Figure 3: Plot of the variation in the number of cells (or the log density of cells) before and after exposure to SWCNT

Thus this study would help in understand whether the E. coli cells are more protected from SWCNT in biofilm than in suspension.

Appendix 1: Protocol for E. coli culture and Plate count

(Need to obtain the protocol from Yu Yung)

Equipment and material required (Tentative):

1. Biofilm reactor (Available in teaching lab)
2. E. coli strains (To be obtained from Yu Yong)
3. Chemical for preparing media for E. coli (Get it from teaching lab, if not found ask Yu Yong for some)
4. Petri plates, filters and agar (Available in teaching lab)
5. A tank to collect waste from the biofilm reactor
6. Incubator, centrifuge and autoclave (Available in teaching lab)
7. Single walled carbon nano tubes

Action items and Timelines (Tentative):

Week 1: (Beginning Monday 03/24/08)

1. Obtain the E. coli bacteria strains and protocol for E. coli culture and plate count. Also learn the safe ways of handing the coli form.
2. Order single walled carbon nano tubes from Sigma – Aldrich. (Web address: http://www.sigmaaldrich.com/catalog/search/ProductDetail/ALDRICH/652512)
3. Download MSDS from Sigma- Aldrich (Completed)
4. Read the biofilm reactor manual and study how to setup it up.
5. Prepare media for E. coli culture
6. Inoculate the strains obtained from Yu Yong and culture E. coli.
7. Do a cell count of the E. coli in the culture to establish the plate count method.

Week 2 and week 3: (Beginning Monday 03/31/08)

1. Set the bioreactor with the media and the culture and the outlet tank and allow the biofilms to form on the coupons.
2. Monitor media flow rate, temperature and other parameters.
3. Conduct a plate count test of the cells in suspension and in biofilm before exposure to SWCNT.
4. Prepare SWCNT solution and establish the setup for pumping it into the biofilm reactor.
5. Remove one rod (with three coupons) each day (and a portion of the cells in suspension) after that to do a cell count of E. coli after exposure to SWCNT.