Removal of bacteria from stormwater using biochar

As more and more urban regions experience urbanization due to rural migration, impervious surface coverage increases, and in turn reduces natural infiltration of stormwater into soil. As a result, urban regions experience depletion in groundwater recharge, increased flooding, increased erosion, and contamination of surface waters receiving overland flow of stormwater. Stormwater contains a myriad of contaminants including suspended solids, nutrients, heavy metals, hydrocarbons, and pathogens. Thus, there is an urgent need to improve management of urban stormwater. This research aims to examine the capacity of biochar; a charcoal-like solid material generated during the pyrolysis of biomass, to remove fecal indicator bacteria (*E. coli*) from stormwater, and the effect of natural organic matter (NOM) on the removal capacity of biochar using a batch experiment. Three types of biochar are used: a commercial biochar, and two biochars produced in a laboratory by pyrolyzing wood chips at 350°C, and 700°C. Batch experiments were conducted in triplicates by adding sand and biochar (5% by weight) in 20 mL of synthetic stormwater containing *E. coli* with or without NOM, and mixed for an hour before analyzing for aqueous concentration of *E. coli.* Experimental results indicates that sand removes 41± 9% of bacteria, the commercial biochar removes 66±5 %, the biochar pyrolyzed at 350°C removes 96 ±0%, and the biochar pyrolyzed at 700°C removes 95±3 % of bacteria. Absence of NOM in stormwater did not change the bacteria concentration significantly. Overall, the biochar pyrolyzed at 700°C seems to be more effective than other biochars in removing bacteria from stormwater.

Method/Procedure:

Growth of bacteria

1) Measure 20 mL of TSB solution and pour into 50 mL centrifuge tube

2) Sterilize a loop with a burner before and after use

3) Take one bacteria colony, where bacteria was streaked a day before, using the loop and place into the 20 mL TSB solution in a 50mL centrifuge tube.

4) Place the tube of *E.coli* in the 37 ^{o}C Incubator (37°C Constant Temperature Room) on the VWR orbital shaker. Make sure the tube's cap is slightly loose to allow oxygen to enter.

5) Take the tube of *E. coli* out of the 37°C Incubator within 14-15 hours

Batch Experiment

1) Initial Preparation
    a. Isolate bacteria with PBS (2x) and with SSW solution (2x)
        1. Take 1 mL of *E. coli* and add to a 1.5 mL centrifuge tube
        2. Centrifuge tube at 9000 rfc for 5 minutes
        3. Dispose of saturate portion in tube

4. Add 1mL of PBS and repeat steps 2 and 3 (2x)
5. Add 1mL of SSW and repeat steps 2 and 3 (2x)

b. Weight Biochar
   1. 5% Sonama biochar: 0.1 g
   2. 2.5% USDA 350°C biochar: 0.05 g
   3. 2.5% USDA 700°C biochar: 0.05 g
c. Weight Sand
   1. 95% Sand: 1.9 g
   2. 97.5% Sand: 1.95 g
   3. 97.5% Sand: 1.95 g
d. Weight 100% Sand- 2.0 g (1 Batch)
e. Add weigh portion(s) into 50mL centrifuge tube
f. Prepare dilution(s) tube
   1. Add 900 uL of PBS into each 1.5 mL centrifuge tube

2) Add 0.3 mL of bacteria sample to SSW solution of 300 mL (bacteria sample concentration is 10^9) for desired initial concentration of 10^6.

3) Stir the SSW solution on the burner at Speed 7 for 5 min

4) Add 20mL of SSW solution to each 50mL centrifuge tube

5) Shake each tube at Speed 5 for 1 hour using the S-500 Orbital Shaker

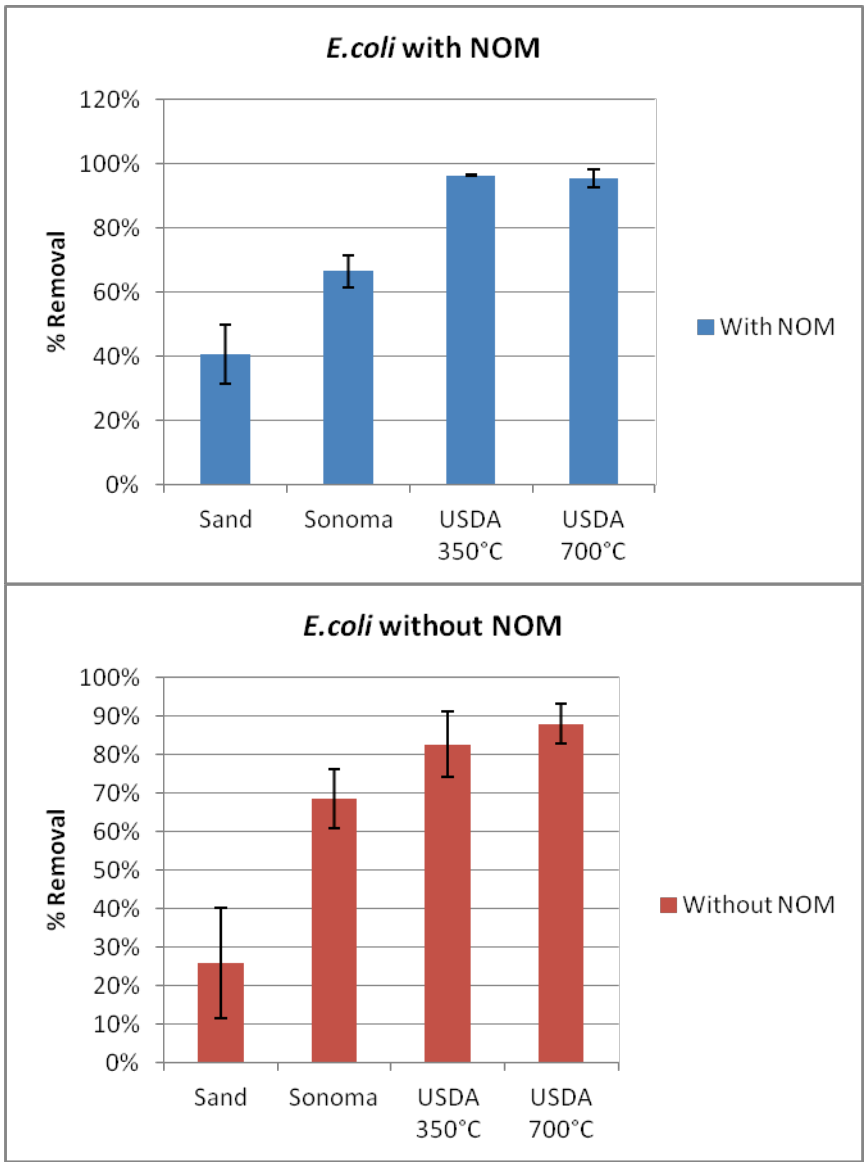
6) Centrifuge each tube at 200 rcf for 5 min

7) Draw 100 uL of each sample and begin dilution

   a. Add the 100 uL sample into the prepared 1.5 mL centrifuge tube
   b. Shake tube 3 times on the Vortex mixer
   c. Draw 100 uL from the 1.5 mL centrifuge tube and add to the next 1.5 mL centrifuge tube
   d. Repeat step c for lower dilutions

8) After dilution, plate the desired dilution(s)

Results:


E.coli with NOM
120%
100%
80%
60%
40%
20%
0%
% Removal
Sand
Sonoma
USDA 350°C
USDA 700°C
With NOM
E.coli without NOM
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
% Removal
Sand
Sonoma
USDA 350°C
USDA 700°C
Without NOM