CHAPTER 3

METHODOLOGY

3.1 Materials and apparatus

3.1.1 Sediment sample

Sediment sample was collected from a canal that runs through Suan-thonburirom park. All debris was removed and sediment sample was then air dry for 2 nights. After that, sediment sample was dried in the oven at 70°C. Dried sediment was ground and sieved through a U.S.standard sieve No.14 (1.4 mm) before used (Figure 3.1). Dried-ground and sieved sediment was analyzed to determine HCB background concentration and spiked with HCB to represent as HCB contaminated for the experiments. The organic matter content of dried-ground and sieved sediment was 11.35%.

Figure 3.1 Dried-ground and sieved sediment sample

3.1.2 Granular sludge seed

Granular sludge seed used for dechlorination was brought from UASB (Upflow Anaerobic Sludge Blanket) Wastewater treatment plant at Serm Suk Public Co., Ltd. with percent dried solid to wet solid of 11.46 (Aditsuda *et al*, 2002). Granular sludge seed was ground and

sieved through a U.S. standard sieve No.50 (0.297 mm.) for homogenization.

Figure3.2 Homogenized granular sludge

3.1.3 Reactor (test-tube),

20-ml syringes were used as reactor for experiment, (Figure 3.3). There were15 syringes for each set of dechlorination experiment (for 5 sampling times and triplicate for each time). Each one was sacrificed for analysis HCB at specific time point.

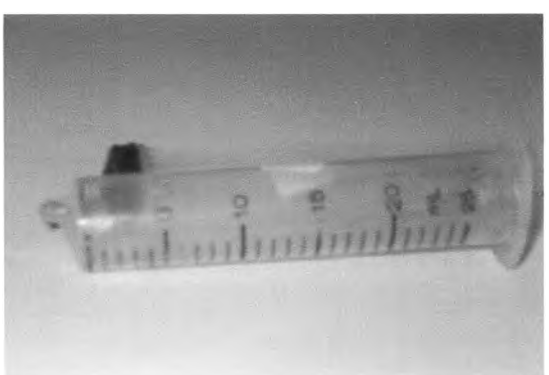


Figure 3.3 test tube (syringe)

3.1.4 Nutrient media

Nutrient media used in all experiment was prepared after Speece, (1996) as shown in Table 3.1.

Table 3.1 Composition of inorganic nutrient media for anaerobic studies

Constituent	Concentration in reactor (mg/l)
NH_4Cl	400
$MgSO_4.7H_2O$	400
$CaCl_2.2H_2O$	50
KCl	400
$(NH_4)_2HPO_4$	80
$FeCl_{2\cdot4}H_2O$	40
$CoCl_{2\cdot6}H_2O$	10
KI	10
$(NaPO_3)_6$	10
$MnCl_{2\cdot4}H_2O$	0.5
NH_4VO_3	0.5
$CuCl_{2\cdot2}H_2O$	0.5
$ZnCl_2$	0.5
$AlCl_{3\cdot6}H_2O$	0.5
$NaMoO_{4\cdot2}H_2O$	0.5
Na_2SeO_3	0.5
$NaHCO_3$	6000

3.1.5 Chemicals

Hexachlorobenzene with 99% analytical standard was purchased from Fluka Chemical Industrial.

95% n-hexane, solvent for organic residue analysis, was purchased from J.T.Baker, Mexico.

Yeast extract, glucose, ethanol, formic acid, lactic acid, acetic acid, butyric acid, and propionic acid were purchased from Standard Lab Company Limited.

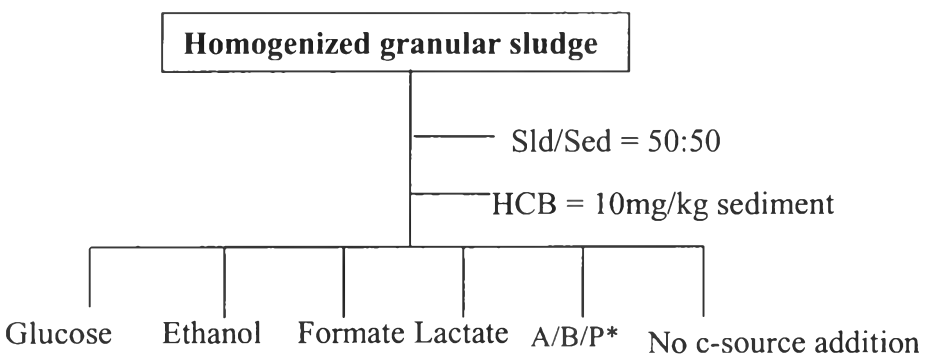
3.1.6 Gas chromatograph.

An electron capture detector (ECD) gas chromatograph (Agilent 6890N) equipped with a HP-5 capillary column (inner diameter, 0.2 mm and length 25 m) was used for HCB analysis.

3.2 Experimental design

3.2.1. The role of carbon-source:

Figure 3.4, six sets of experiment (15 syringes of each set) was performed in order to examine the role of carbon source. Five groups of carbon sources were mixed with nutrient media solution to serve as electron donors in order to enhance the dechlorination process. The rate of HCB reduction was compared among these five groups of substrates and controlled with no c-source addition. The sludge to sediment ratio and HCB concentration were consistent for all 6 sets of experiment.



Note; * is the combination of acetic acid, butyric acid and propionic acid with 2:1:1 (based on COD) ratio.

Figure 3.4 Diagram for the role of carbon source supplementation experiment.

Ninety 20-ml syringes containing 5 g dried-ground and sieved sediment was applied by 5 ml of 10 ppm HCB/Toluene solution into every single syringe (spiking of sediment). After toluene evaporation overnight, 10-ml of nutrient media solution containing 300 mg/l of yeast extract and different types of carbon sources was filled into every syringe, which provided the same COD concentration approximately 3000 mg/l (3 g/l of glucose, 1.44 g/l of ethanol, 8.64 g/l of formic acid, 2.8 g/l of lactic acid, and the combination of 1.4 g/l of acetic acid, 0.42 g/l of butyric acid and 0.5 g/l of propionic acid). Five g. (wet weight) of homogenized sludge seed was added into each syringes while

continuously sparging with deoxygenated N_2 and CO_2 (80:20) for 1 minute then put a septum into syringe with minimal headspace in order to keep in anaerobic condition. The mixture of sediment, sludge and nutrient media became sediment slurry.

3.2.2 The role of Sludge:Sediment ratio *with* and *without* c-source

From Figure 3.5, four sets of experiment were performed.

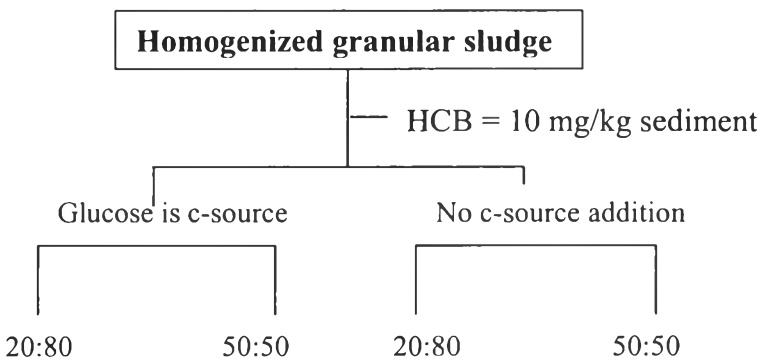


Figure 3.5 Diagram of sludge to sediment ratio experiment

Eight grams of contaminated sediment and 2 grams of homogenized sludge were used. Eight ml of 10 ppm HCB/Toluene solution was applied to the sediment. Ten ml of nutrient media solution with 300 mg/l of yeast extract and glucose (3000 mg/l COD) was filled.

3.2.3 Control condition

The sediment slurry for control set was performed as same as the first experiment group but no carbon sources were added. Besides, yeast extract was added to control with yeast extract set but not in control without yeast extract set to observe the effect of yeast extract to microbial mechanism (Figure 3.6).

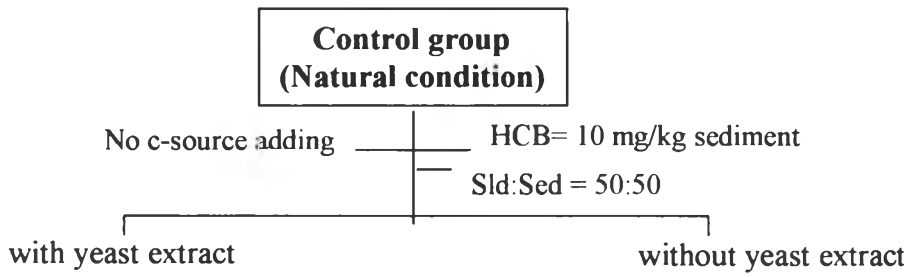


Figure 3.6 Diagram of control condition

Figure 3.7 showed all test-tube in one set of experiment. They were kept in the darkness at room temperature. The sediment slurry was hand-shaken once a day.

Figure 3.7 One set of experiment, 5 times of sampling with triplicates each.

3.3 Time of sampling

The samples were collected at week 0,2, 4, 6, and 9. First samples were collected immediately after setting up of experiment was completed. All collected samples were frozen at 4°C in order to cease microbial activity. The amount of gas production was recorded before analysis. Figure 3.8 showed the sample of after 6 weeks of the experiment, septum was moved up.

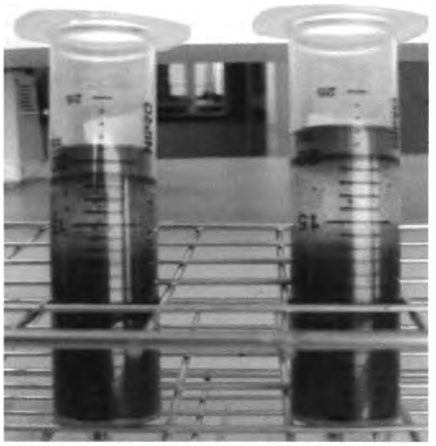


Figure 3.8 Septum was moved up by gas produced from microcosm.

3.4 Extraction method

The entire sediment slurry from each syringe was withdrawn to 50-ml Erlenmeyer flask and rinsed the syringe and septum thoroughly with 20 ml of n-hexane. The sample was then shaken by orbital rotary shaker at 170 rpm for 2 hours. Afterward, n-hexane layer was withdrawn. The second and third extraction were done by adding fresh10 ml of n-hexane and done in the same manner as the first extraction.

The extract (n-hexane layer) was filtered to remove all particles through membrane filter with 0.45 μm pore size and adjusted filtered sample to 20 ml of total volume with n-hexane before injecting to gas chromatography.

3.5 GC analysis

All quantification was performed with external standard. A volume of 1 μl of each sample extract was injected under the following conditions; Temperature injector: 150°C, temperature detector: 250°C, initial column temperature: 150°C hold for 1 min then, programmed at 150°C to 200°C at a rate of 35 °C /min and 200°C to 275°C at a rate of 40°C/min and hold for 3 min. The carrier gas was helium (average linear volume of 30ml min-1) and the make up gas was N_2 at 70ml min-1. Split ratio was kept at 100:1.