EFFECT OF BIOFILM ON FORMATION AND REDUCTION OF DISINFECTION BY-PRODUCTS

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ผลกระทบของไบโอฟิล์มต่อการเกิดขึ้นและการลดลงของสารพลอยได้จากการฆ่าเชื้อโรค

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วัตถุประสงค์หลักของงานวิจัยนี้ได้แก่การศึกษาการนำไปใช้และการดูดซับสารพลอยได้จากกระบวนการฆ่าเชื้อโรคโดยไบโอฟิลม์ทั้งที่ถูกยับยั้งและไม่ถูกยับยั้งกิจกรรมทางชีววิทยา รวมถึงศักยภาพการเกิดสารพลอยได้จากการฆ่าเชื้อโรคของไบโอฟิลม์ในระบบจ่ายน้ำประปา เพื่อที่จะทำความเข้าใจชะตากรรมและการเคลื่อนที่ของสารพลอยได้จากการฆ่าเชื้อโรคที่เกิดจากไบโอฟิลม์ในระบบจ่ายน้ำประปา การศึกษาการนำไปใช้ของสารพลอยได้จากการฆ่าเชื้อโรคที่เป็นตัวแทนของสารสี่กลุ่มหลัก (คอโลฟอร์ม (CF) ไดคลอโรอะซิติกแอซิด (DCAA) ไดคลอโรอะซิโตไนไตรล์ (DCAN) และ1,1 ไดคอโลโพรพาโนน (1,1 DCP) ดำเนินการภายใต้การทดลองแบบทีละเทและควบคุมค่าพีเอสที่ 7 จากผลการทดลองพบว่า DCAN เป็นสารที่สามารถถูกนำไปใช้โดยกิจกรรมทางชีววิทยามากที่สุด ตามด้วย 1,1 DCP ดังนั้นปรากฏการณ์นี้อาจทำให้ความเข้มข้นของสาร DCAN และ 1,1 DCP ลดลงในระบบจ่ายน้ำประปา อย่างไรก็ตาม CF และ DCAA สามารถถูกดูดซับได้โดยพื้นผิวของไบโอฟิลม์

ศักยภาพการเกิดสารพลอยได้จากการฆ่าเชื้อโรคของไบโอฟิลม์และสารพวกโพลิเมอร์ที่อยู่ภายนอกเซลล์ (EPS) ดำเนินการภายใต้ระยะเวลากักเก็บ 7 วันเพื่อที่จะได้รับศักยภาพการเกิดสารพลอยได้จากการฆ่าเชื้อโรคแต่ละชนิดสูงสุด คาร์บอนจากเซลล์มีศักยภาพการเกิดสาร CF สูงกว่า EPS และคาร์บอนจากเซลล์ในไบโอฟิลม์น่าจะเป็นแหล่งคาร์บอนหลักในการเกิดสารกลุ่มฮาโลคีโตน (HKs) ซึ่งโดยรวมแล้วสารพลอยได้จากการฆ่าเชื้อโรคชนิดคาร์บอนมีศักยภาพในการเกิดขึ้นสูงกว่าและมีแนวโน้มที่เกิดสารพลอยได้จากการฆ่าเชื้อโรคที่มีจำนวนแฮโลเจนอะตอมสูง

สาขาวิชา การจัดการสิ่งแวดล้อม ลายมือชื่อนิสิต .....

ปีการศึกษา 2557 ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

ลายมือชื่อ อ.ที่ปรึกษาร่วม .....

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KITTITHACH LIMTRAKUL: EFFECT OF BIOFILM ON FORMATION AND REDUCTION OF DISINFECTION BY-PRODUCTS. ADVISOR: ASSOC. PROF. PATIPARN PUNYAPALAKUL, Ph.D., CO-ADVISOR: PARINDA THAYANUKUL, Ph.D., 87 pp.

The main objective of this study is to investigate the DBPs reduction and abiotic control by active and inactive biofilm as well as DBPs formation potential of biofilm in water distribution system in order to understand fate and transportation of DBPs that might be occurred by biofilm in water distribution system. The biofilm used in study was collected from water reservoir in Bangkok metropolitan area. The reduction and abiotic control of four representatives of main DBPs groups (chloroform (CF), dichloroacetic acid (DCAA), dichloroacetonitrile (DCAN) and 1,1 Dichloro-1-propanone (1,1 DCP)) were conducted by batch experiment under controlling pH at 7. The obtained results shown that DCAN is the most biological consumable followed by 1,1 DCP, therefore it might cause the reduction of DCAN and 1,1 DCP concentration in water distribution system. However, CF and DCAA were slightly consumed by physical attachment on biofilm surface.

DBPs formation potential of biofilm and extracted extracellular polymeric substance (EPS) were conducted by chlorination for 7 days to obtain the maximum formation potential of each DBPs species. Cellular carbon might have higher potential to form CF than the carbon source from EPS. HAAs formation potential has closer relation to the carbon source from EPS than carbon from cellular source. And cellular carbon source of biofilm might be the main carbon source for HKs formation potential. Carbonaceous DBPs had higher formation potential and had a trend to produce higher halogenic DBPs.

Field of Study:	Environmental Management	Student's Signature .....
		Advisor's Signature .....
Academic Year:	2014	Co-Advisor's Signature .....

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CHAPTER 1
INTRODUCTION

1.1 Introduction

Providing safety for drinking water is the most significant thing in public health issue, therefore water supplying has to provide drinking water without pathogenic microorganism. Treated water is transported to an end user at long distance from the starting point. Drinking water treatment plant has to disinfect the product water by using chlorine or chloramines before delivering water because the disinfection potency still remains in distribution pipeline. If there is some leakage in distribution system, the chlorine residual can protect the water safety from the microbial contamination (Lautenschlager et al., 2014)

Due to the accessibility of drinking water distribution system, understanding of the microbial ecology in drinking water distribution system is restricted. Maintaining of biological stability in drinking water distribution system is abundantly challenging because of less nutrient, high pressure and high concentration of oxidant. However, many research found that there were some microbial cultures were able to grow in that environment. Microorganisms had better chance survival attached to the internal wall of distribution pipeline and 95% of attached microorganisms produced biofilm (Douterelo et al., 2014).

Disinfection by-products (DBPs) in drinking water have been concerned for a long period of time due to their potential to cause crucial adverse effect to human body (e.g. cancer and reproductive effect). DBPs became one of significant parameters for drinking water regulations, research, and treatment system since the discovery of them in the early 1970s (W. Chu et al., 2012).

Carbonaceous DBPs such as Trihalomethanes (THMs) and Haloacetic acids (HAAs) are widely regulated for the maximum concentration limits in many countries; however, Nitrogenated DBPs including Halonitromethanes (HNMs) and Haloacetonitriles (HANs) which mostly have higher toxicity than the carbonaceous

DBPs have not yet been regulated. Moreover, there precursor sources are not well understood.(Prarat, 2011)

Formation of DBPs is not only occurred in chlorination process of drinking water treatment plant due to the reaction between chlorine and natural organic matters, but also occurred in drinking water distribution system where residual chlorine reacts with the bacterial biofilm or their extra cellular polymeric substance (EPS) (Wang et al., 2012). Moreover, Biofilm and EPS contain polysaccharides and protein which can be a precursor of carbonaceous and nitrogenous DBPs. Besides, DBPs itself may be the nutrient source for biofilm. The DBPs fate and formation potential related to the biofilm in drinking water distribution system are not well identified.

1.2 Objective of the study

1. To investigate DBPs formation potential of biofilm and EPS in drinking water distribution system.
2. To investigate the DBPs reduction by biological activity of biofilm in drinking water distribution system.

1.3 Hypothesis

1. Biofilm and extracellular polymeric substances (EPS) in water distribution system can interact with chlorine and form DBPs.
2. Biofilm is able to reduce DBPs by its own biological activities

1.4 Scope of the study

1. Biofilm will be obtained from a tap water reservoir in Bangkok area.
2. Extracellular polymeric substances will be extracted from biofilm and investigated the composition.
3. Reduction experiment will be done under completely mix of active biofilm and DBPs in batch system.
4. DBPs Formation potential experiment will be done under excess chlorine residual for 7 days to investigate the maximum formation potential.

5. DBPs Formation potential of EPS and biofilm will be investigated.
6. Four different group of DBPs as following will be studied.
    3.1 Trihalomethane (THM)
        3.1.1 Chloroform (CF)
    3.2 Haloacetic acids (HAAs)
        3.2.1 Chloroacetic acid (MCAA)
        3.2.2 Dichloroacetic acid (DCAA)
        3.2.3 Trichloroacetic acid(TCAA)
    3.3 Haloacetonitriles (HANs)
        3.3.1 Chloroacetonitrile (MCAN)
        3.3.2 Dichloroacetonitrile (DCAN)
        3.3.3 Trichloroacetonitrile (TCAN)
    3.4 Haloketones (HKs)
        3.4.1 1,1 Dichloro-2-propanone (1,1-DCP)
        3.4.2 1,1,1 Trichloropropanone (1,1,1 TCP)
7. DBPs will be measured by gas chromatography with electron capture detector.

CHAPTER 2
LITERATURE REVIEWS

2.1 Disinfection By-Products

Disinfection by-products in drinking water are complexes of organic and inorganic halogenated compounds. The major halogenated DBPs are chlorinated DBPs, which are mainly occurred by a reaction between organic compounds and free chlorine or chloramine (disinfectant) in the water (W. Chu et al., 2013; W. H. Chu et al., 2009; Prarat, 2011).

The proportional amount of DBPs in drinking water were shown in figure 2.1

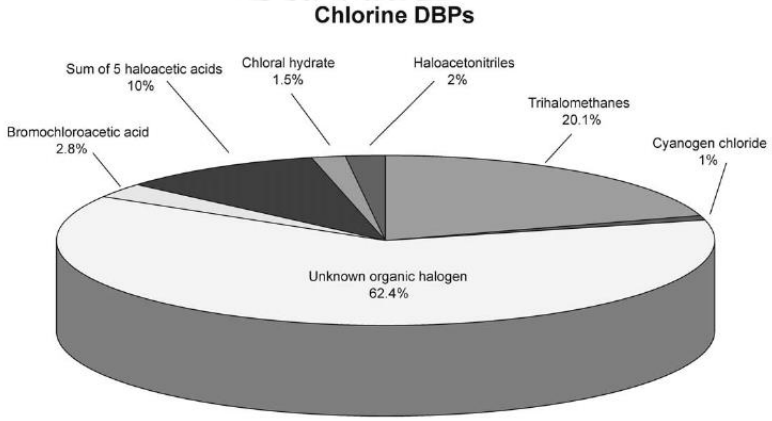


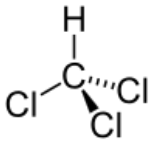
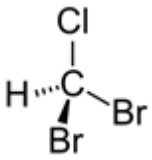
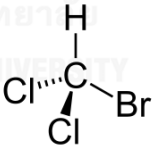
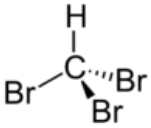
Figure 2.1 Relatives amount of DBPs in chlorinated drinking water (Richardson, 2003).

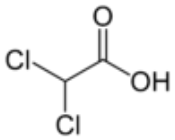
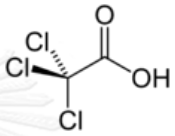
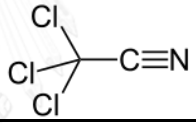
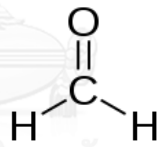
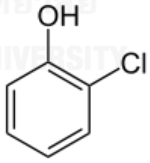
Several carbonaceous disinfection by-products (C-DBPs) were recognized as carcinogens such as trihalomethanes (THMs) and haloacetic acids (HAAs). Many countries regulated them with the maximum limit in drinking water. However, there are much less studies for halogenated nitrogenous disinfection by-products (N-DBPs) such as haloacetonitriles (HANs) and halonitromethanes (HNMs) that may be regulated in the future because of their higher toxicity than C-DBPs (Gopal et al., 2007; Prarat, 2011).

Although the DBPs presents in drinking water at low concentration, the risk of adverse health effect should not be overlooked because of the high exposure in daily

life such as drinking, washing, and cooking. There are various health effects as shown in Table 2.1

Table 2.1 Classification of disinfection by-products and their health effect

Classification	Compound	Molecular Structure	Health effect
Trihalomethanes (THMs)	Chloroform		USEPA classified Chloroform as a Carcinogenic Group B2. Bladder, Rectal, Colon cancer and marked fatty cyst formation in the liver
	Dibromochlorometh-ane		USEPA classified Dibromochloromethane as a Carcinogenic Group C. Hepatic lesions, Liver cancer, Nervous system and reproductive effect
	Bromodichlorometh-ane		USEPA classified Bromodichloromethane as a Carcinogenic Group B2. Kidney cancer, Renal cytomegaly and reproductive effect
	Bromoform		USEPA classified Bromoform as a Carcinogenic Group B2. Cancer liver, kidney and reproductive effects Hepatic lesions

Classification	Compound	Molecular Structure	Health effect
Haloacetic acids (HAAs)	Dichloroacetic acid (DCAA)		USEPA classified DCAA as a Carcinogenic Group B2. liver cancer, Lesions observed in the testes, cerebrum, cerebellum, Liver and reproductive effect
	Trichoroacetic acid (TCAA)		USEPA classified TCAA as a Carcinogenic Group C. Liver cancer, Hepatocellular necrosis
Haloacetonitirle	Trichloroacetonitrile		Cancer, mutagenic and clastogenic effects
Halogenated aldehydes and ketones	Formaldehyde		Mutagenic
Halophenols	2-Chlorophenol		Reproductive effect, Cancer and tumor promoter

Source: USEPA IRIS at:http://cfpub.epa.gov/ncea/iris/index.cfm?fuseaction=iris.showSubstanceList

2.2 Disinfection in drinking water system

2.2.1 Drinking water treatment plant

Drinking water supply is one of public utility which is important for our daily life (Hebert et al., 2010). Raw water quality is very significant for the water supply because safety of consumer health, efficiency of treatment process and effectively of treatment designing depend mostly on initial characteristic of raw water. Raw water should have high quality in order to save cost of treatment operation. The characteristics of precursor natural organic matters (NOM) in raw water also effect to

types and formation potential of DBPs when react with chlorine. Higher dissolve organic nitrogen (DON) level in raw water, such as amino acid and polypeptide, trends to increase nitrogenous DBPs yield of total formation potential.

Precursor NOM is a main problem for water supply engineering. NOM has been defined as a major source of DBPs formation in drinking water. However, the treatment of NOM has been limited by the technical development, they cannot be absolutely eliminated and consequently they pass to the distribution system.

Providing safety for drinking water is the most significant thing in public health issue, therefore water supplying has to provide drinking water without pathogenic microorganism. Treated water is transported to an end user at long distance from the starting point. Drinking water treatment plant has to disinfect the product water by using chlorine or chloramines before delivering water because the disinfection potency still remains in distribution pipeline. If there is some leakage in distribution system, the chlorine residual can protect the water safety from the microbial contamination (Lautenschlager et al., 2014).

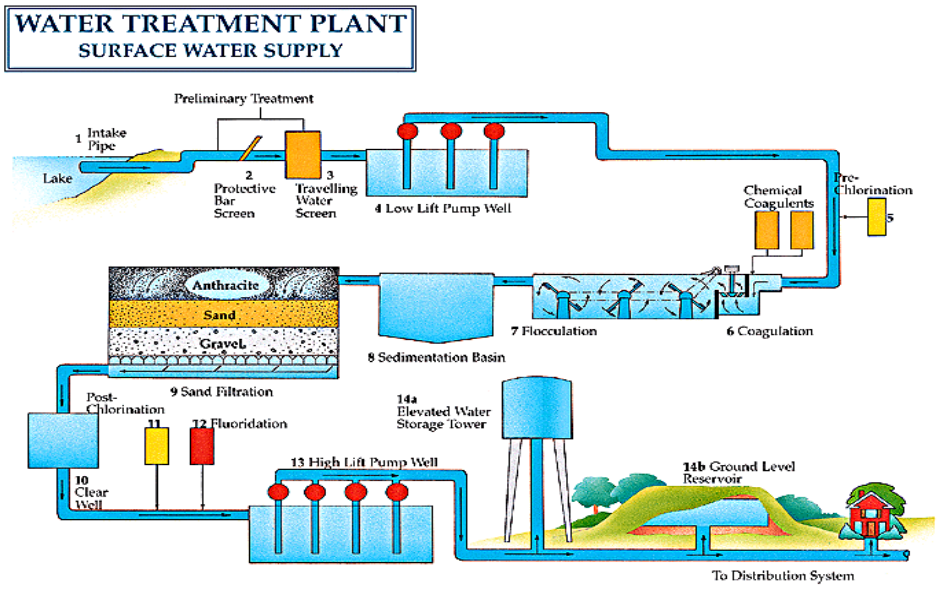


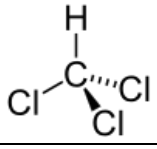
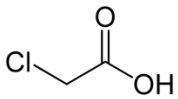
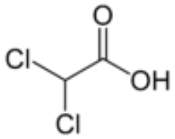
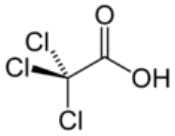
Figure 2.2 Traditional drinking water treatment diagrams.
Source: Canadians opposed to fluoridation at:http://cof-cof.ca/surface-water-treatment-plant-flow-diagram/

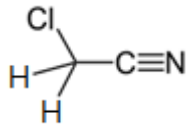
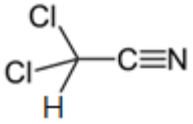
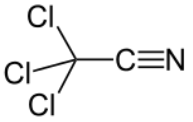
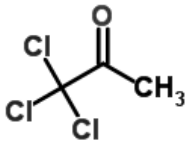
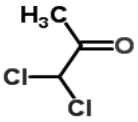
The typical water supply treatment process is shown in Figure 2.2 Chlorination process is occurred twice in the treatment line. First is pre-chlorination process where chlorine added directly to the raw water at the retention pond for eliminating algae. Algae introduce several problems in the later treatment processes; for example, algae that grows in sedimentation tank causing the gravity settling problem in sedimentation basin and clogging the filter media in sand filtration tank. Moreover, algae are one of culprits that cause foul taste and odor in drinking water. Second is post-chlorination for ensuring that pathogens were disinfected at the chlorination tank before storing water in clear well.

2.2.2. Disinfection by-product formation

As we know that the disinfection by chlorine in a drinking water system is an adoring process, the DBP formation is unavoidable. Natural organic matter in the system will interact with chlorine and turn into chlorine base-form of disinfection by-products in both carbonaceous and nitrogenous DBPs (Table 2.2).

Table 2.2 Physicochemical characteristics of some DBPs

Classification	Compound	Molecular Structure	Boiling point (°C)	Molecular weight (g)	Density (g/cm^3)
Trihalomethanes (THMs)	Chloroform (CF)		61.2 °C	119.38	1.48
Haloacetic acids (HAAs)	Chloroacetic acid (MCAA)		189.3	94.50	1.58
	Dichloroacetic acid (DCAA)		194	128.94	1.5634
	Trichoroacetic acid (TCAA)		197	163.39	1.63

Haloacetonitriles (HANs)	Chloroacetonitrile (MCAN)		126	75.5	1.193
	Dichloroacetonitrile (DCAN)		112	109.94	1.369
	Trichloroacetonitrile (TCAN)		84	144.39	1.44
Haloketones (HKs)	1,1,1-Trichloroacetone (1,1,1-TCA)		161.41	161.41	1.475
	1,1-Dichoropropanone (1,1-DCP)		117.3	126.97	1.291

2.3 Microbiology in drinking water distribution system

Due to the arduous accessibility of drinking water distribution system, understanding of the microbial ecology in drinking water distribution system is restricted. Maintaining of biological stability in drinking water distribution system is abundantly challenging because of less nutrient, high pressure and high concentration of oxidant. However, many research found that there were some microbial cultures were able to grow in that environment. Microorganisms had better chance survival attached to the internal wall of distribution pipeline and 95% of attached microorganisms produced biofilm (Douterelo et al., 2014).

2.3.1 Extracellular polymeric substances (EPS)

Extracellular polymeric substances (EPS) are defined as polymeric compounds, which are present outside of cell and among microbial aggregates that are not adjacent to the cell membrane layer (Sheng, G.P. et al., 2010). Normally, EPS are

high molecular weight secretions. They absorb organic matters and products of cell activities such as cellular hydrolysis of macromolecules in the water.

The common function of the EPS is the aggregation of bacterial cells and the formation of biofilm clinging on the surface pipe wall of distribution system. Moreover, in very limited nutrient environment, EPS have the significant role in enhancing NOM consumption and retention for bacterial cell. Many studies found that EPS provide binding sites for nutrients to permit the absorption of organic compound. Moreover, in condition of nutrient shortage bacterial cell can use a part of EPS as carbon or energy sources (Sheng et al., 2010).

2.3.2 EPS structure

The EPS can be divided into two types which are soluble EPS (Soluble macromolecules, slimes, and colloids) and bound EPS (attached organic materials, condensed gels, sheaths, loosely bound polymers, and capsular polymers). Practically, these two types of EPS are able to be separated by centrifugal technique because soluble EPS are weakly adhered to cell (soluble EPS are able to dissolve into solvent), while bound EPS are tightly adhered to cell. Since the interaction between soluble EPS and bacterial cell is very weak, the study on soluble EPS is limited. Many studies in this field mentioned specifically to the bound EPS.

Bound EPS can be separated into two layers, tightly bound EPS and loosely bound EPS. Tightly Bound EPS is the inner layer that is bound tightly and stably with the cell surface while loosely bound EPS is the outer layer, which is the dispersible slime layer (Sheng et al., 2010).

2.3.3 EPS composition

The chemical compositions of EPS consists of polysaccharide, protein, humic substances, and nucleic acids, but polysaccharides and proteins (including derivatives such as lipopolysaccharides, glycoproteins, and lipoproteins) are usually found as a major composition of EPS (75-90%)(Sheng et al., 2010).

2.3.3.1 Polysaccharides

Polysaccharides are one of the major compositions of the EPS. The bacterial cells secrete neutral carbohydrate monomer and uronic acids (such as manuronic, glucuronic, and galacturonic acid). Type of secreted monomer is the key of specification nature (neutral, cationic, or anionic) and type of macromolecule. EPS Polysaccharides are classified to homopolysaccharides (such asdextran, curdlan, and cellulose) and heteropolysaccharides (such as, alginate, xanthan gum, gellan gum, welan gum, colanic acid, K30-antigen, and hyaluronic acid)(Sheng et al., 2010).

2.3.3.2 Protein

Proteins are one of the major components of EPS as same as polysaccharides. Protein in EPS presents as enzyme and structural protein. In EPS matrix several enzymes have been found. The extracellular enzymes which are secreted by bacterial cells act like water soluble and insoluble substances, which is trap organic particles. Structural proteins, such as the cell surface-associated and extracellular carbohydrates binding with protein, assist bacterial cells to build polysaccharides matrix network linking between bacterial surface and extracellular surface. Glycoproteins, which are generated when sugar molecule covalently attached on protein, were reported to assist the cell for maintaining stability such as improving structural integrity and stability of signaling and inter-cellular communication. Generally, EPS hydrophilic content is mainly due to polysaccharides, protein, and DNA molecules, while the hydrophobic properties are related to polysaccharides-linked to methyl and acetyl group (More et al., 2014).

2.3.3.3 Extracellular DNA

EPS of most bacteria contains extracellular DNA such as extracellular deoxyribonucleic acids. Amount of produced extracellular DNA is depended on the type of bacterial species (More et al., 2014).

2.3.3.4 Lipids and surfactant

Lipids, lipopolysaccharides, and derivatives lipids are composed in the EPS matrix as well. Lipopolysaccharides assist in adhesion to surface. Bio-surfactant such as surfactin, viscosin, and emulsion is help to dissolve hydrophobic substances in media. This is important for bacterial attachment and detachment from oil droplet. Moreover, bio-surfactant can have antibacterial and antifungal properties (More et al., 2014).

2.3.4 Bacteria culture in drinking water distribution system.

Drinking water distribution system is quite complicated and inaccessible system. The system mainly consists of pipes, vessels, fittings, and valves that are made from variety of different materials such as cast iron, polyvinylchloride (PVC), and polyethylene, so the variety and density of attached microbial culture on the pipe surface are different. Moreover, some studies found that the shear force which is applied to surface pipe due to hydraulic movement affected to thickness and density of biofilm (Douterelo et al., 2014).

Douterelo et al. (2014) investigated the bacterial community in an operational flushing pipe in Northwest England using 16s rDNA sequences analysis, and the result are shown in the Figure 2.3. In plastic pipe mostly found *Sopirochaeta* and *Methylobacterium*, but in cast iron pipe *Lynsinibacillus* and *Acidobacterium* is mostly found. M.M. Williams simulated the drinking water distribution system for 7 years. The cycling of drinking water in simulated distribution system is 1 day. He found that bacterial cultures in the system consisted of α-proteobacteria 51.4%, $_{\gamma}$-proteobacteria 4.1%, cyanobacteria 4.1%, and Firmicutes 2.7%. The Phylogenetic tree were formed with software PAUP* and shown in Figure 2.4.

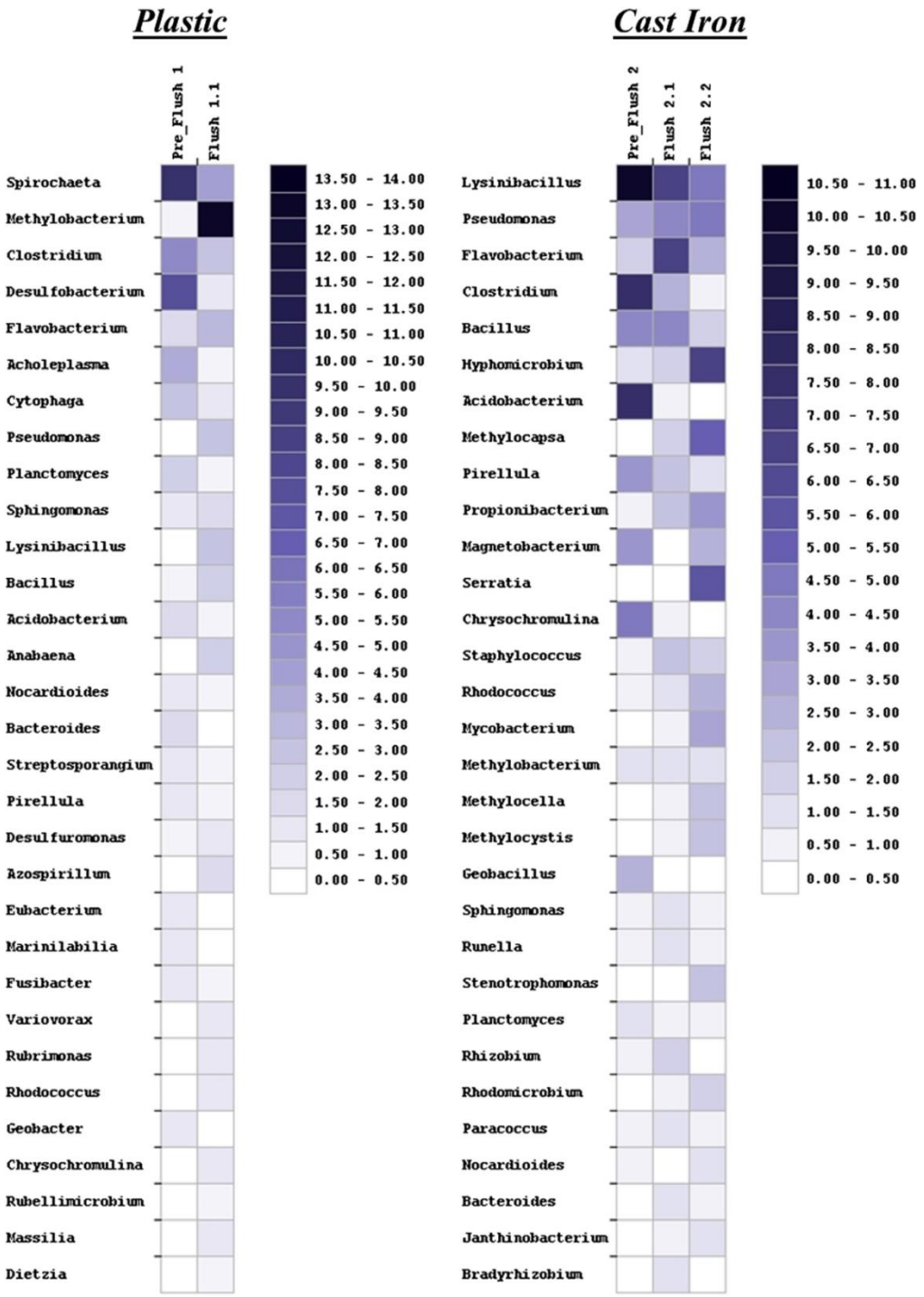


Figure 2.3 Heatmaps showing the percentages of the most abundant species at genus level within water samples from plastic and cast iron pipes.

The relative abundance has been calculated as the average of 4 biological replicates for every sample (Douterelo et al., 2014).

Figure 2.4 Phylogenetic analysis of bacterial 16S rDNA in chlorinated Cincinnati distribution water

2.4 EPS and DBPs Formation potential

Wang et al. (2013) studied the DBPs formation potential by using surrogate chemical of EPS monomer composition of *Pseudomonas aeruginosa, Pseudomonas putida,* and biofilm samples obtained from a water utility. The solutions were chlorinated with 20 mg-Cl/mg-C dose at pH 7. The DBPs formation potential was analyzed by GC electron captured detector. The EPS components of the biofilm samples in this study are shown in the, Table 2.3, 2.4, 2.5 and 2.6.

Table 2.3 Show the protein monomer proportion of EPSs composition.

Amino acid composition	Molecular weight (Da)	P. aeruginosa EPS (%)	P. Putida EPS (%)	Biofilm EPS (regrown) (%)	Biofilm EPS (isolated) (%)
Asx	115.1/133.1	6.4	8.8	6.0	13.0
Glx	129.1/147.1	6.6	8.7	13.6	12.9
Ser	87.1	2.7	3.9	3.1	8.0
His	137.2	0.8	1.5	0.6	0.7
Gly	57.1	11.1	12.7	12.1	14.7
Thr	101.1	3.8	4.6	4.8	7.1
Ala	71.1	20.4	15.4	19.3	11.6
Arg	156.2	3.9	5.6	2.6	1.2
Tyr	163.2	3.6	2.7	2.9	1.2
Val	99.1	8.0	7.5	8.4	2.8
Met	131.2	3.6	1.8	2.8	1.2
Phe	147.2	2.9	3.2	2.9	2.2
Ile	113.2	5.2	4.2	5.7	4.9
Leu	113.2	9.6	8.7	7.7	6.2
Lys	128.2	4.6	5.8	2.5	1.6
Pro	97.1	7.1	4.2	4.9	5.5

Abbreviations: Asx- asparagine/aspartic acid; Glx- glutamine/glutamic acid; Ser- serine; His- histidine; Gly- glycine; Thr- threonine; Ala- alanine; Arg- arginine; Tyr- tyrosine; Val- valine; Metmethionine;Phe- phenylalanine; Ile- isoleucine; Leu- leucine; Lys- lysine; Pro- proline. Biofilm EPS (regrown): EPS was extracted from mixed species biofilm regrown in $1/10^{th}$ LB broth.
Biofilm EPS (isolated): EPS was directly extracted from mixed species biofilm isolated from a water utility. Proportion of protein monomer was analyzed by HPLC technique.

Table 2.4 The saccharine monomer proportion of EPSs composition.

Group of Polysaccharide monomer	Polysaccharide monomer	Molecular weight (Da)	P. aeruginosa EPS (%)	P. Putida EPS (%)	Biofilm EPS (regrown) (%)	Biofilm EPS (isolated) (%)
Sugar mine	D-glucosamine	179.2	22.0	27.2	83.6	81.1
Uronic acid	D-glucuronic acid	194.1	31.0	33.8	15.1	5.9
	D-galacturonic acid	194.1	0.3	0.0	0.0	0.0
Hexose	D-glucose	180.6	0.8	0.0	1.3	12.3
	D-mannose	180.2	3.5	0.0	0.0	0.0
	D-arabinose	150.1	3.8	0.0	0.0	0.7
	L-rhamnose	164.2	0.0	7.2	0.0	0.0
Pentose	L-fucose	164.2	38.6	27.4	0.0	0.0
	D-ribose	150.1	0.0	4.4	0.0	0.0

Biofilm EPS (regrown): EPS was extracted from mixed species biofilm regrown in 1/10th LB broth. Biofilm EPS (isolated): EPS was directly extracted from mixed species biofilm isolated from a water utility. Proportion of protein monomer was analyzed by HPLC technique.

Table 2.5 DBPs Yields of Grouped Amino Acids of Extracted EPS

Aminoacid group	Extracted EPS	DBP Yield (µg/mgC)								
		THM	HAA			HAN		HK		HNM
		CF	MCAA	DCAA	TCAA	DCAN	TCAN	1,1-DCP	1,1,1-TCP	TCNM
Acidic	P.aeruginosa EPS	21.7 ± 4.0	15.3 ± 4.5	624.9 ± 98.8	27.7 ± 7.1	13.0 ± 0.5	0	0.2 ± 0.1	0.8 ± 0.1	0.5 ± 0.1
	P. putida EPS	11.9 ± 0.5	8.4 ± 5.6	573.3 ± 19.5	15.3 ± 2.8	10.1 ± 3.6	0	0.2 ± 0.1	0.4 ± 0.1	0.4 ± 0.2
	Biofilm EPS (regrown)	14.1 ± 1.7	8.8 ± 0.9	492.8 ± 79.6	9.1 ± 2.6	8.8 ± 1.0	0	0.2	0.5 ± 0.1	0.5 ± 0.1
	Biofilm EPS (isolated)	15.5	8.0 ± 2.1	534.4 ± 61.7	13.9 ± 0.5	8.6 ± 2.3	0	0.1	0.2	0.3 ± 0.1
Basic	P.aeruginosa EPS	11.3 ± 0.2	7.2 ± 0.7	44.3 ± 3.6	10.4 ± 1.9	2.2 ± 0.4	0	0	0.7	0.3
	P. putida EPS	12.2 ± 1.5	8.1 ± 0.1	53.9 ± 0.8	13.4	2.4 ± 0.3	0	0	0.7	0.4
	Biofilm EPS (regrown)	13.5 ± 4.2	7.5 ± 1.4	46.4 ± 3.4	14.1 ± 8.5	2.0 ± 0.3	0	0	0.7 ± 0.1	0.5 ± 0.3
	Biofilm EPS (isolated)	17.6 ± 0.6	2.3 ± 0.4	82.1 ± 0.1	12.7 ± 1.9	1.7 ± 0.3	0	0.2	0.6 ± 0.1	0.6 ± 0.1
Aliphatic	P.aeruginosa EPS	4.3 ± 0.7	2.6 ± 0.1	8.4 ± 0.3	4.5 ± 0.1	0.9 ± 0.1	0	0.2	1.0 ± 0.1	0.1
	P. putida EPS	6.9 ± 0.4	3.4 ± 0.8	11.2 ± 2.3	10.2 ± 5.0	0.8	0	0.3	1.1	0.2 ± 0.1
	Biofilm EPS (regrown)	9.4 ± 3.1	2.7 ± 0.5	9.9 ± 0.4	8.2 ± 2.9	1.0 ± 0.2	0	0.3	1.3 ± 0.2	0.3
	Biofilm EPS (isolated)	11.8 ± 0.3	2.9 ± 0.1	11.6 ± 1.2	8.5 ± 0.1	0.1	0	0.1	0.5	1.0
Aromatic	P.aeruginosa EPS	28.4 ± 1.0	9.2 ± 0.7	47.7 ± 10.8	75.2 ± 9.4	2.2	0.2 ± 0.1	0.2	0.6 ± 0.1	0.6 ± 0.1
	P. putida EPS	28.4 ± 0.6	10.9 ± 1.1	42.1 ± 0.6	65.8 ± 2.8	1.6 ± 0.1	0	0.2	0.5 ± 0.1	0.6
	Biofilm EPS (regrown)	32.1 ± 1.6	10.7 ± 3.1	51.6 ± 4.3	80.2 ± 5.7	2.2 ± 0.8	0.3 ± 0.1	0.2	0.6	0.7 ± 0.1
	Biofilm EPS (isolated)	21.5	3.6 ± 0.8	21.8 ± 0.4	32.2 ± 1.7	1.0	0.2 ± 0.1	0.2	0.2 ± 0.1	0.6 ± 0.1
Polar uncharged	P.aeruginosa EPS	11.2 ± 2.1	3.9 ± 1.9	65.1 ± 3.1	12.7 ± 2.4	8.8 ± 2.2	0.8 ± 0.3	0	0.4	0.8 ± 0.3
	P. putida EPS	13.7 ± 4.2	6.6 ± 0.2	73.2 ± 3.4	16.5 ± 2.5	10.4 ± 0.5	0.8 ± 0.1	0	0.3 ± 0.1	0.9
	Biofilm EPS (regrown)	15.8 ± 5.0	6.0 ± 1.0	63.3 ± 2.5	19.0 ± 7.6	9.1 ± 0.5	0.6 ± 0.1	0	0.5 ± 0.2	0.8
	Biofilm EPS (isolated)	20.2 ± 1.4	2.8	114.3 ± 5.2	50.1 ± 1.7	10.4 ± 1.5	0.2	0.2	0.4	1.4 ± 0.1

Hydrophobic	P.aeruginosa EPS	18.4 ± 1.9	8.2 ± 0.2	11.8 ± 3.5	15.9 ± 4.6	1.2 ± 0.3	0	0.3	1.5 ± 0.3	0.3
	P. putida EPS	42.0 ± 7.6	9.3 ± 0.3	13.3 ± 3.9	17.9 ± 5.1	3.0 ± 0.4	0	0.3 ± 0.1	3.3 ± 0.2	0.4
	Biofilm EPS (regrown)	15.4 ± 1.5	7.9 ± 0.3	15.0 ± 0.9	20.5 ± 1.0	0.9 ± 0.1	0	0.3	1.2 ± 0.2	0.2
	Biofilm EPS (isolated)	8.0 ± 0.2	3.4 ± 1.1	10.8 ± 0.2	12.9 ± 1.0	0.1	0	0.1	0.3	1.0 ± 0.2

Table 2.6 DBP Yields of Polysaccharide Monomers of Extracted EPS

Polysaccharide monomer groups	Extracted EPS	DBP Yield (μg/mgC)								
		THM	HAA			HAN		HK		HNM
		CF	MCAA	DCAA	TCAA	DCAN	TCAN	1,1-DCP	1,1,1-TCP	TCNM
Pentose	*P.aeruginosa EPS*	17.7 ± 2.4	10.5 ± 0.3	14.1 ± 0.1	7.9 ± 0.4	0	0	0.3	0.3 ± 0.1	0
	P. putida EPS	21.0 ± 2.8	8.4 ± 5.6	18.8 ± 1.6	12.2 ± 1.1	0	0	0.3	0.2	0
	Biofilm EPS (regrown)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	Biofilm EPS (isolated)	17.4 ± 2.0	9.4 ± 1.2	16.1 ± 2.6	8.4 ± 1.0	0	0	0.2	0.2	0
Hexose	P.aeruginosa EPS	32.7 ± 0.5	5.2 ± 1.4	23.2 ± 2.6	10.8 ± 2.4	0	0	0.4	0.7	0
	P. putida EPS	36.0 ± 4.0	14.4 ± 1.2	29.4 ± 2.7	20.8 ± 1.2	0	0	0.3	0.5	0
	Biofilm EPS (regrown)	19.0 ± 1.3	2.1	20.9 ± 1.2	8.8 ± 1.9	0	0	0.4 ± 0.1	0.2	0
	Biofilm EPS (isolated)	21.4 ± 2.1	2.8 ± 1.0	21.9 ± 0.2	8.3 ± 1.1	0	0	0.4 ± 0.1	0.3	0
Uronic acid	P.aeruginosa EPS	20.4 ± 5.3	8.5 ± 2.7	30.0 ± 5.2	16.6 ± 0.2	0	0	0.2 ± 0.1	0.3	0
	P. putida EPS	21.0 ± 1.1	5.0 ± 2.3	33.1 ± 0.9	19.3 ± 3.9	0	0	0.3 ± 0.1	0.3 ± 0.1	0
	Biofilm EPS (regrown)	24.0 ± 5.3	7.7 ± 1.5	29.7 ± 5.7	18.3 ± 2.6	0	0	0.3 ± 0.1	0.4 ± 0.1	0
	Biofilm EPS (isolated)	24.8 ± 4.2	6.0 ± 3.8	29.1 ± 4.8	21.1 ± 1.4	0	0	0.4	0.4 ± 0.1	0
Amino sugar	P.aeruginosa EPS	21.5 ± 4.4	5.4 ± 0.4	23.8 ± 2.9	12.9 ± 3.1	0.4 ± 0.2	0	0.2 ± 0.1	0.5 ± 0.3	0.5 ± 0.1
	P. putida EPS	22.5 ± 3.0	5.8 ± 0.5	27.6 ± 2.5	10.7 ± 6.3	0.5 ± 0.2	0	0.3	0.5 ± 0.2	0.5 ± 0.1
	Biofilm EPS (regrown)	23.7 ± 4.8	5.1 ± 0.3	27.5 ± 2.6	10.2 ± 5.6	0.4 ± 0.1	0	0.3	0.5 ± 0.2	0.5 ± 0.1
	Biofilm EPS (isolated)	25.9 ± 1.8	5.5 ± 0.9	25.7 ± 0.1	14.6 ± 0.7	0.5 ± 0.1	0	0.3 ± 0.1	0.7 ± 0.1	0.7

The result of DBP formation potential (Table 2.4.3 and 2.4.4) showed that protein components trended to have higher DBP formation potential than saccharide components. Relatively high levels of N-DBPs were observed in the high organic nitrogen component such as proteins (Wang et al., 2013).

Pseudomonas Putida, which EPS mainly consists of proteins, had the highest HAAs and CF formation potential yield due to the highest protein content. Moreover, *Pseudomonas Putida* had higher DBPs formation potential than *Pseudomonas aeruginosa* which its EPS mainly consists of polysaccharide (Wang et al., 2013).

From the previous study we knew that there are microorganisms in drinking water distribution system and their effect on DBPs formation in pipe system. However, effects of those attached microorganism (biofilms) and their extracellular polymeric substance (EPS) on DBPs formation potential (DBPs-FP) in Thailand is still unclear. Moreover, the removal of DBPs related to the biological activity of biofilm in drinking water distribution system is still needed to be investigated.

CHAPTER 3
MATERIAL AND METHOD

3.1 Chemical

1. Disinfection by-products
   1.1 Trihalomethane
      - Chloroform
   1.2 Halo acetic acids
      - Chloroacetic acid
      - Dichloroacetic acid
      - Trichloroacetic acid
   1.3 Halo acetonitriles
      - Chloroacetonitriles
      - Dichloroacetonitriles
      - Trichloroacetonitriles
   1.4 Halo ketones
      - 1,1 Dichloro-2-Propanone
      - 1,1,1 TrichloroPropanone
2. Chemical agent used in this research
   - H_2SO_4
   - $CuSO_4$
   - $NaSO_4$
   - $NaHCO_3$
   - NaHOCl
   - Methanol
   - Methyl butyl ether
   - BCA protein test kit (cell signaling)
   - Phosphate buffer pH 7, ionic strength 0.01M (0.5795 g and KH_2PO_4 1.00435 in 1L of deionized water)

3.2 Apparatus

- Gas Chromatography (Agilent GC6890, Electron Capture Detector)
- Carbon analyzer (Shimadzu VCPH, Solid and Liquid analyzer)
- pH meter
- Magnetic stirrer
- Shaker
- Balance

3.3 Biofilm

Biofilm in this study was obtained from tap water reservoir using spatula. The sample was placed in to the glass test tube with phosphate buffer (pH 7, ionic strength = 0.01 M). The obtained biofilm was flaky orange. Figure 3.1 show the obtained biofilm in this study.

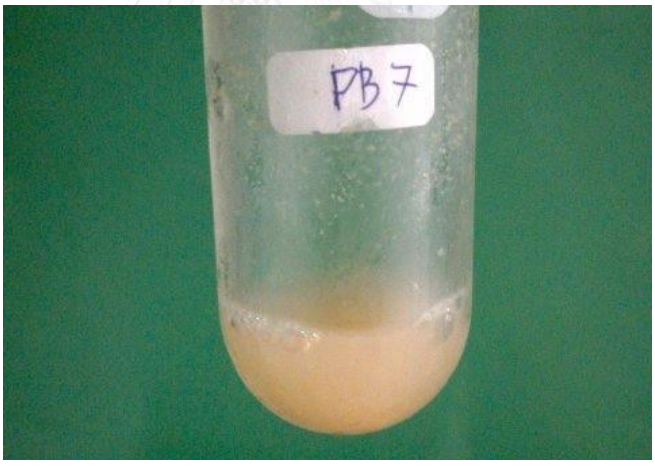


Figure 3.1 Biofilm from drinking water reservoir

3.4 Biofilm characterization

- The carbon content of biofilm was quantified by solid total organic carbon analyzer (TOC)
- Total solid, total volatile solid and total fixed solid was measured by heating at 550 ^{o}C for three hours to quantify overall composition between organic and inorganic content in dried biofilm. The chart of measurement was shown in figure 3.2.

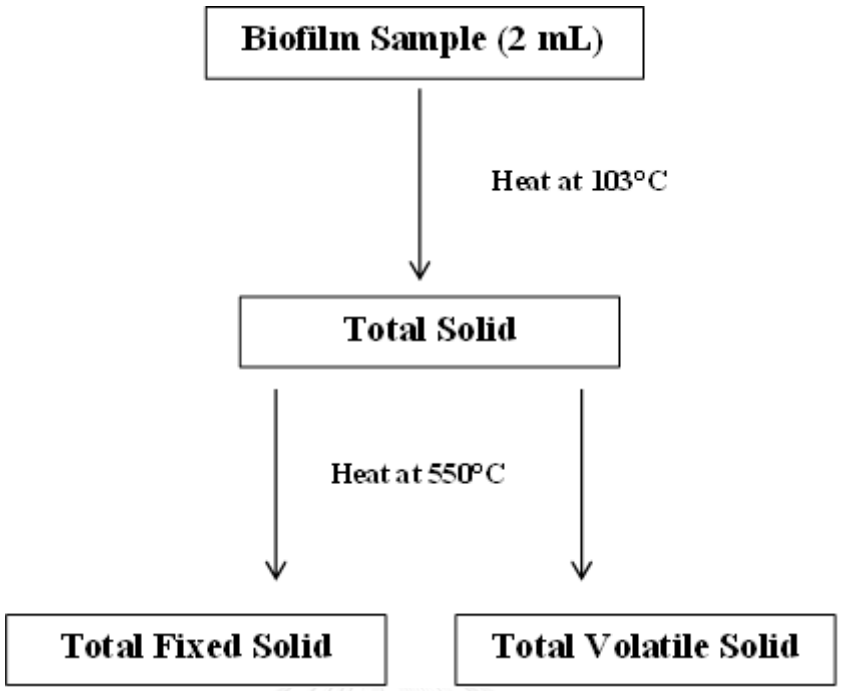


Figure 3.2 Total solid, total fixed solid and total volatile solid characterization

Total solid, total fixed solid and total volatile solid were calculated from equation below.

$$\text{Total solid} = \frac{\text{Weight of container after 103 °C heating} - \text{initial weight of container}}{\text{Volume of biofilm sample}}$$

$$\text{Total fixed solid} = \frac{\text{Wieght of container after 103 °C heating} - \text{Weight of container after 550 °C heating}}{\text{Volume of biofilm sample}}$$

$$\text{Total volatile solid} = \text{Total Solid} - \text{Total fixed solid}$$

3.5 EPS extraction procedure

This study preferred to use physical extraction method because chemical extraction using organic solvents effect on the result of DBPs formation potential.

A 100 ml of completely mixed biofilm was placed in 250-mL glass beaker. 120 W of ultrasound was performed for 2 minutes, and then the solution was centrifuged 20000 rpm for 20 minutes. The upper liquid was obtained for DBPs formation potential. The EPS was quantified by solid TOC. The EPS will be measured protein by BCA protocol and polysaccharide by Dubois method.

3.6 EPS characterization

3.6.1 Protein content of EPS

Protein content of EPS was analyzed by BCA assay test kit (Cell signaling technology, micro plate scale)

Standard curve of colorimetric method was performed for protein measurement. Reagent A, Reagent B and buffer solution was use for color activation of sample and measure at 540 nm. The detail of procedure was followed handout instruction. The packaging of applied test kit for measuring protein content is shown in Figure 3.3.

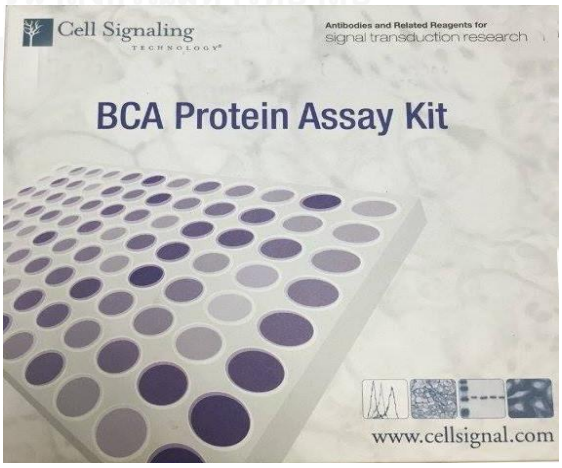


Figure 3.3 Cell Signaling BCA protein assay test kit

3.6.2 Saccharide content of EPS

Saccharide content of EPS was analyzed by Duboise method. The procedure of saccharide determination method was show in figure 3.4.

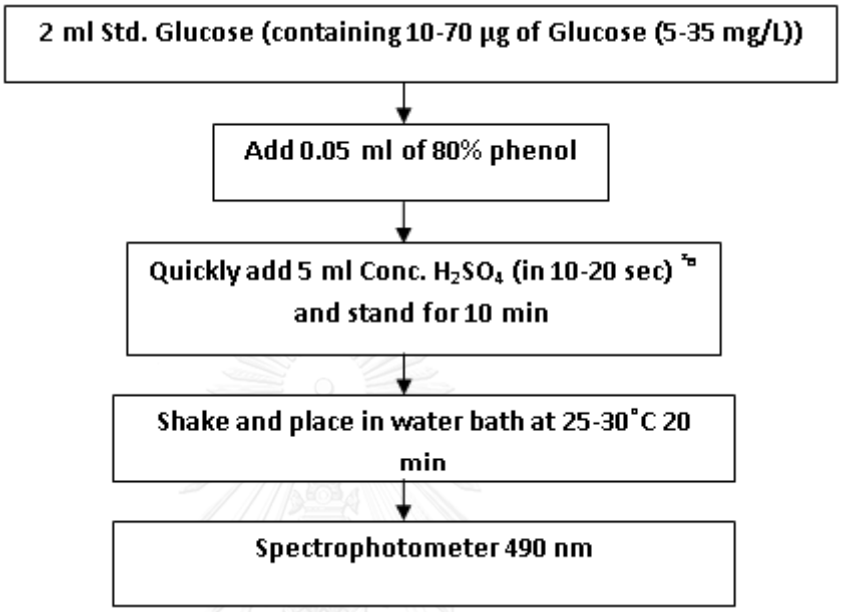


Figure 3.4 Saccharide content determination procedure

3.7 Inactivated biofilm preparation

Active biofilm was sterilized by using autoclave at 110 ºC for 15 minutes and the biofilm was quantified by TOC machine.

Plate count technique was applied for checking the microbial activities.

3.8 Disinfection by-products reduction by biological activity experiment

DBPs (HAAs, HANs, THMs, and HKs) 100, 300 and 500 µg/L in 40 ml phosphate buffer (pH 7, ionic strength 0.01M) solutions was prepared in flask with glass stopper. Active or inactive biofilm 50 mg/L will be added for each solution followed by incubation at room temperature in shaker at 200 rpm. After 24 hours, each sample was collected and filtrated with 0.45 µm filter. DBPs measurement followed the modified USEPA method 511.1 and 522.2. The experimental process is shown in figure 3.5.

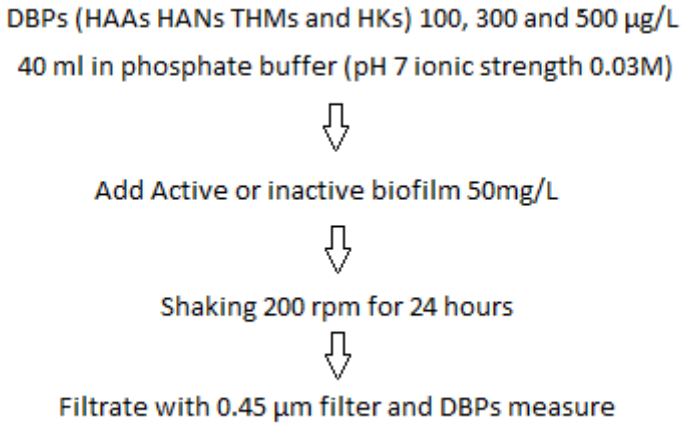


Figure 3.5 DBPs reduction by biological activity experiment

3.9 Disinfection by-products formation potential of biofilm

The 10 mg/L (final concentration) of either active biofilm was inoculated into 250 ml phosphate buffer solution (pH 7, ionic strength 0.01M). Each solution was chlorinated using 20 mg-Cl/mg-C of biofilm under head space-free condition and shaking at 200 rpm for 7 days. After chlorination process, the sample was collected and was filtered through 0.45 µm membrane. DBPs measurement followed the modified USEPA method 511.1 and 522.2.

3.10 Disinfection by-products formation potential of EPS

The 10 mg-C/L of EPS was performed into250 ml phosphate buffer solution (pH 7, ionic strength 0.01M). Each solution was chlorinated at 20 mg-Cl/mg-C of EPS under head space-free condition and shaking at 200 rpm for 7 days. After chlorination process the sample was collected and was filtered through 0.45 µm membrane. DBPs measurement followed the modified USEPA method 511.1 and 522.2.

3.11 Disinfection by-products analytical methods

Gas chromatography technique equipped with electron capture detector (GC-ECD) was adapted to quantify the amount of DBPs at very low concentration.

3.3.1 Modified USEPA Method 511.1

This method was modified from USEPA 511.1 (Prarat, 2011) and used for qualify THMs, HANs and HKs. The 30 mL of sample was placed into 40 mL amber glass vial with polypropylene screw cap and PTFE septum. For each sample, 5 g of anhydrous sodium sulfate and 2 mL methyl-tert-butyl ether (MtBE) were added in order to increase the aqueous matrix ionic strength. The vial was shaken for 2 minutes and transfers the upper layer (MtBE layer) into 2 mL GC vial. The organic layer (1 µL) was injected into an Agilent GC6890 gas chromatograph equipped with an electron capture detector (GC/ECD). The condition and parameter of gas chromatography will be performed as shown in the table 3.1 and the extraction procedure was shown in the Figure 3.6.

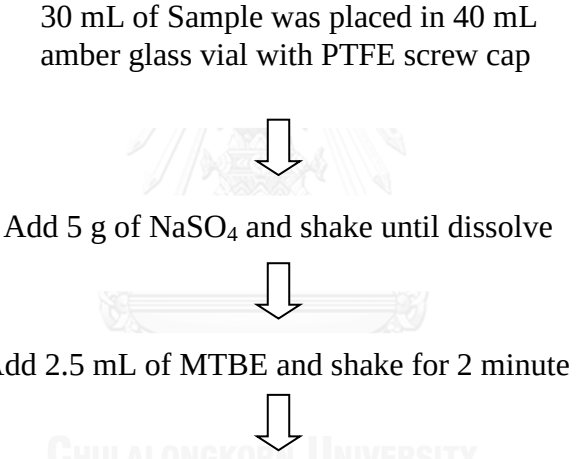
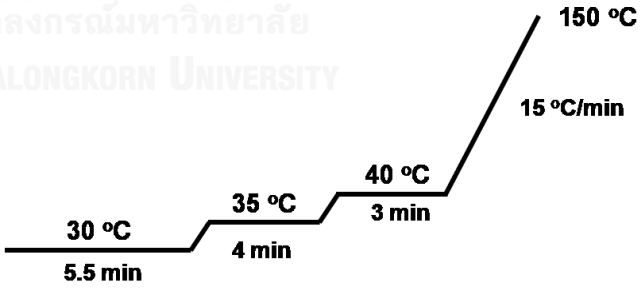


Figure 3.6 Extraction procedure of modified USEPA method 511.1

Table 3.1 Gas chromatography condition and parameters for modified USEPA Method 511.1

Helium-carrier gas flow rate	25 cm sec^{-1}
Nitrogen-carrier gas	Use as a make-up gas
Detector type	Micro electron capture detector (ECD)
Column	Fused silica capillary column (VF-X Varian,30 m x 0.32 mm i.d. x 0.25 μm film thickness)
Injector temperature	200 ^{o}C
Injection type	Splitless mode
ECD Detector Temperature	300^{o}C
Oven programed condition	[1] initial temperature of 30 ^{o}C for 5.5 min [2] ramping to 35 ^{o}C at 10 ^{o}C min^{-1} and holding for 4 min [3] ramping to 40 ^{o}C at 10^{o}C $min^{-1}$and holding for 3 min [4] ramping to 150 ^{o}C at 15^{o}C min^{-1}. 

3.3.2 Modified USEPA Method 522.2

This method was modified from USEPA 522.2 (Prarat, 2011) and used for qualify HAAs. This analytical method is divided into 2 steps as following:

1. Derivatization step

A 15 mL of sample was placed in to the 40 mL amber glass vial with the polypropylene screw cap and PTFE faced septum and added 25 µL of surrogate standard 2,3-dibromopropionic acid (50 µg/L in MtBE solution) into each sample. The sample was added by 0.5mL of concentrated sulfuric acid and 4 g of $NaSO_4$and shaken until dissolve. After $NaSO_4$ dissolved, 2.5 g of $CuSO_4 \cdot 5H_2O$ was added and shaken until dissolve and 2.5 mL of MTBE was added. The derivatization procedure was shown in the Figure 3.7.

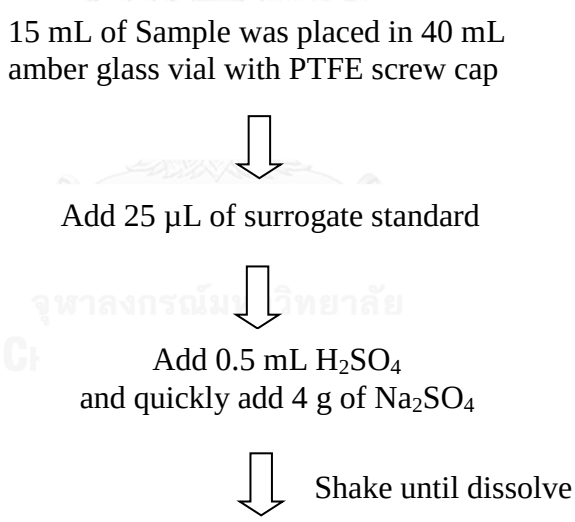


Figure 3.7 Derivatization procedure for modified USEPA method 522.2

2. Methylation step (Extraction step)

A 1.625 mL MtBE layer (organic layer) was transferred to 40 mL amber glass vial containing 2 mL of 10 percent sulfuric in methanol, and then transfer the vial into water bath at 50°C for 2 hours. After incubation, the vial was cool-down using refrigerator at 4°C for 3 minutes. The $NaHCO_3$ was added into cool sample, and then the vial was shaken carefully because this step had to release the carbon dioxide gas frequently. After shaking, organic layer was transferred to 2 mL GC vial. The sample was always analyzed in 24 hours after extraction. The condition and parameters of gas chromatography were performed as shown in the table 3.2 and the extraction procedure was shown in the Figure 3.8.

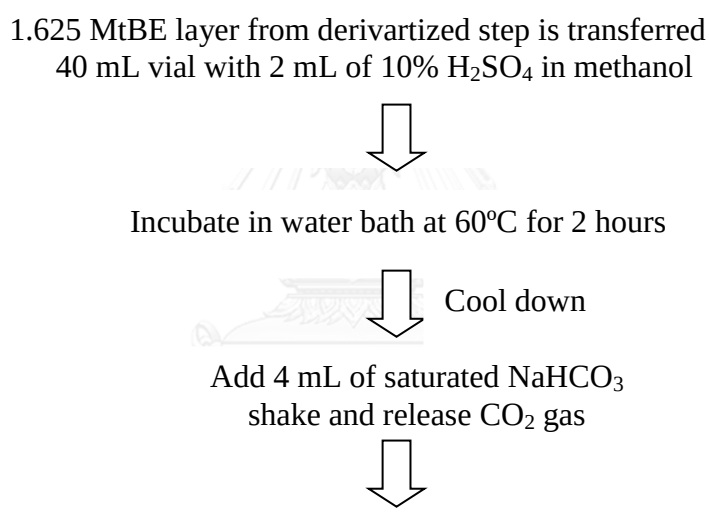
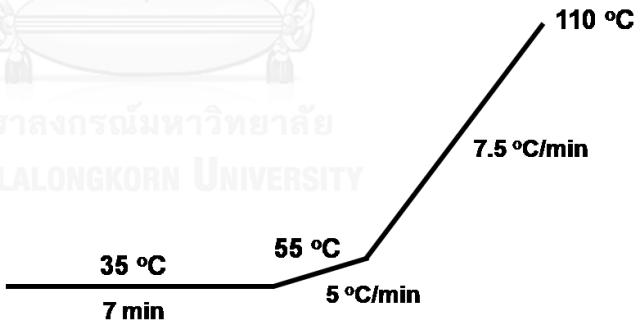


Figure 3.8 Extraction procedure for modified USEPA method 522.2

Table 3.2 Gas chromatography condition and parameters for modified USEPA Method 522.2

Helium-carrier gas flow rate	25 cm sec^{-1}
Nitrogen-carrier gas	Use as a make-up gas
Detector type	Micro electron capture detector (ECD)
Column	Fused silica capillary column(HP-1,30 m x 0.32mm i.d. x 0.1µm film thickness)
Injector temperature	200 ^{o}C
Injection type	Splitless mode
ECD Detector Temperature	290^{o}C
Oven programed condition	[1]initial temperature of 35^{o}C for 7 min, [2] ramping to 55^{o}C at 5^{o}C min^{-1},  [3] ramping to 110^{o}C at 7.5^{o}C min^{-1}

CHAPTER 4
RESULT & DISCUSSION

4.1 Biofilm characterization

In this study, biofilm was obtained from residential drinking water reservoir and placed into test tube with pH 7 phosphate buffer (0.01M ionic strength). The biofilm was re-suspended in the test tube before using for characterization. The biofilm mixture was analyzed total solid content to quantify the amount of biofilm that was used in each characterization and further experiments. Moreover, total carbon content (TOC in solid sample) analyzed by solid total carbon analyzer was applied to identified organic composition (carbon base) of biofilm suspension.

Furthermore, extracellular polymeric substance (EPS) in suspended biofilm was extracted by physical extraction method (ultrasonic technique). Then, total carbon content (TOC) of extraction EPS solution was analyzed to quantify amount of EPS that can be extracted by applied method. Moreover, obtained EPS solution was measured protein content by BCA assay, including saccharide content, in order to analyze the EPS composition. The obtained data was used in quantitative analysis for DBPs abiotic control and reduction as well as DBPs formation potential of biofilm and extracted EPS. Characterized parameters were concluded in Table 4.1

Table 4.1 The list of biofilm's characterization parameters

Material	Characterization
Biofilm	Total solid (SS)
	Total organic carbon content (TOC) (solid)
	Inorganic content
EPS	Total organic carbon content (TOC) (liquid)
	Saccharide content
	Protein content

4.1.1 Total organic carbon content of biofilm

In this study, biofilm in completely mixed solution was analyzed organic carbon content by solid total organic carbon analyzer (TOC analyzer). Organic carbon content can be referred to organic component of biofilm. TOC of biofilm was analyzed 3 times and the obtained detail was shown in Table 4.2.

The carbon content was used as a considerable parameter for quantifying the amount of biofilm in this research based on organic composition of biofilm and organic precursors for DBPs-FP of each DBPs in biofilm structure can be suggested to be organic content from EPS and cell. Generally, TOC content can measure directly by the equation below.

$$Total\ Carbon\ content = Inorganic\ carbon + Organic\ carbon$$

Table 4.2 Total organic carbon content of biofilm

Biofilm	Total solid (mg/L)	Organic carbon content (g-C/L)[a]	% Organic carbon (%g-C/gTS)[b]	% Organic carbon (%g-C/gTVS)[c]
1	17.82±1.12	2.03±0.45	11.4	38.9
2	21.65±0.89	2.21±0.23	10.2	34.9
3	28.84±1.45	3.11±0.66	10.6	36.2
Average			10.7	36.7
SD			0.4	2.1

a : (g-C/L) = gram carbon per Liter

b : (g-C/gTS) = gram carbon per gram total solid

c : (g-C/gTVS) = gran carbon per gram total volatile solid

Biofilm content consisted of organic carbon approximately 10-11% of total solid by weight and percentage of organic carbon per total volatile solid is 36%. However, comparing with the previous studies, the organic carbon content (%w/w dry mass) of biofilm is barely reported and strongly depend on various component such as microorganisms, EPS, entrapped particles, dissolved substances and etc. The obtained

result from Table 4.2 might be supposed that the organic carbon content (included cell and EPS) is a little bit low this mean that main composition of biofilm layer is not biofilm cell, but it might be inorganic composition such as precipitated calcium and magnesium ions and also entrapped inorganic particles.

4.1.2 Total organic and total inorganic content of biofilm

Volatile organic and fixed inorganic content of biofilm were measured by heating to 550 ^{o}C for three hours to quantify overall composition between organic and inorganic content in dried biofilm. The organic and inorganic content data was shown in the table 4.3.

From the result, biofilm had organic content approximately 29% by weight of total solid. This result indicated that most of biofilm component consist of inorganic compounds which might be caused from the non-precipitate hardness, which is remained form the water treatment process in drinking water.

As same as the result of total organic carbon content of biofilm (section 4.1.1), the organic component of biofilm is lower than inorganic component. However, the presence of organic component is still has high possibility to be the precursor for DBPs-FP and can affect the fate and transportation of occurred DBPs in water distribution system such as DBPs utilization by biofilm as carbon source.

Table 4.3 Total organic and total inorganic content of biofilm

Biofilm	Total solid (mg/L)	Total volatile solid (mg/L)	Total fixed solid (mg/L)	% TVS (%g TVS/g TS)[a]	% TFS (%g TFS/g TS)[b]
1	17.82±1.12	4.83±0.3	12.99±0.82	27.1	72.9
2	21.65±0.89	6.44±0.26	15.21±0.63	29.8	70.3
3	28.84±1.45	8.91±0.45	19.93±1.0	30.9	69.1
Average				29.3	70.8

a : (%g TVS/g TS) = % gram total volatile solid per gram total solid

b : (%g TFS/g TS) = % gram total fixed solid per gram total solid

From the report of Bakke et al. (2001), the ratio between organic carbon of EPS and cellular carbon is 30/70.

4.1.3 Inactivated biofilm

For inactive biofilm, biofilm was deactivated by autoclave at 110℃ for 20 minutes and then the sample was checked for the microbial activity by plate count technique. After 7 days the plate did not has any colony (duplicate), therefore it can be concluded that the biofilm was completely deactivated. Moreover, organic carbon content of biofilm before and after deactivation was shown in the table 4.4. From obtain result, it can be seen that the organic carbon content was not changed significantly due to the deactivation process. And the temperature and time consume in deactivation process were adjusted to be the level that has lowest effect to the structure of macro molecule compound both in cell and EPS.

Table 4.4 Comparison of organic carbon content of active and inactive biofilm

Type of Biofilm	Total solid (g/L)	Organic carbon content (g-C/L)	% Organic carbon (%g-C/gTS)
Biofilm	17.82	2.03	11.39
Inactive Biofilm	18.94	2.10	11.09

4.1.4 Carbon content of Extracted EPS

EPS from biofilm was extracted by ultrasonic technique and quantified by organic carbon content by liquid total carbon analyzer. The organic carbon content and efficiency of EPS extraction were shown in table 4.5. Organic carbon content of extracted EPS can be referred to the amount of DBPs yield in the DBPs formation potential investigation.

Table 4.5 Organic carbon content and efficiency of EPS

Total carbon content of biofilm (mg-C of biofilm/L)	**Total organic carbon content of extracted EPS (mg-C of EPS/L)**	**Efficiency of extraction (% mg-C of EPS / mg-C of biofilm)**
2.21 g-C/L	329.1 mg-C/L	14.89 %

From the EPS extraction study of Adav and Lee (2011) found that the efficiency of ultrasonic extraction was approximately 36% of total organic carbon content of biofilm. And from the report of Bakke et al. (2001), the ratio between organic carbon of EPS and cellular carbon is 30/70. However, the of obtain ratio (between % mg-C of EPS / mg-C of biofilm) in table 4.5 was very low compared with previous study which might be related to the variation of biofilm and the completeness of extraction procedure.

Total organic carbon content of the biofilm is able to be separated into carbon from EPS and carbon from biofilm cell. Hence, proportion of organic carbon content of EPS can roughly refer to proportion of organic content of cell, which is correlated to the total organic content of biofilm. The roughly quantification of carbon content of cell and the estimation of EPS organic proportion in this study was shown in the table 4.6.

Table 4.6 Estimation of EPS and cell ratio

Part of Biofilm	% g Carbon content / g TS	% Total organic carbon / Total Organic content	% weight / TVS of biofilm (%g / g TVS)
EPS	1.52	5.17	35.9
Cell	9.19	31.33	64.1

- 2279 mg/L weight of EPS

4.1.5 Saccharide content of extracted EPS

Saccharide is one of the major components of EPS. This study measured the saccharide content and will be used to discuss about the relationship to DBPs formation potential. Saccharide of EPS is suggested to have strong correlation to the carbonaceous DBPs formation. Figure 4.1 illustrates the calibration curve for measuring the saccharide concentration. And the saccharide content of extracted EPS was shown in the table 4.7.

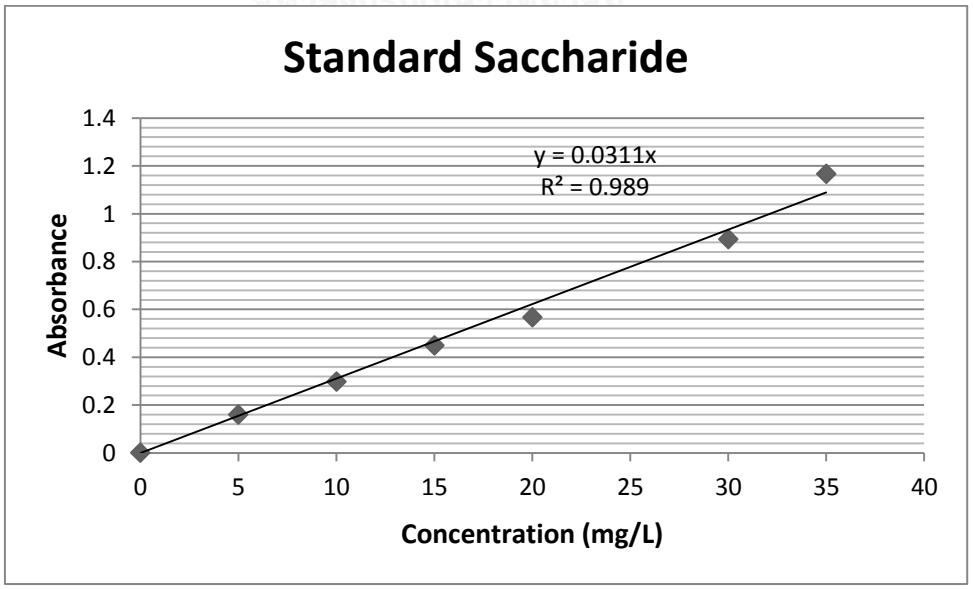


Figure 4.1 The calibration curve for measuring the saccharide concentration

Table 4.7 the saccharide content of extracted EPS

EPS Sample	Absorbance (Abs)	Concentration (mg/L)	% Weight of Saccharide / TVS of biofilm (%g / g TVS)	% Weight of saccharide / TS of biofilm (%g / g TS)
	0.547	175.8	2.77	0.81
	0.54	177.5	2.79	0.82
	0.56	180.1	2.83	0.83
	Average	177.8	2.79	0.82

Saccharide content of the extracted EPS was 177.8 mg/L or 2.79 % (% Weight of Saccharide / TVS of biofilm). Comparing this data to the data of % weight of EPS / TVS of biofilm (%g / g TVS) which shown in table 4.6, it can be seen that the ratio of saccharide to EPS was approximately 2.3%. Although, the ratio of saccharide was low, however, from the study of Wang (2013), they reported that saccharide content correlated to the carbonaceous DBPS formation potential, which are formed in the formation potential test. The type of DBPs depended on the structure and functional group of saccharide. For example, monosaccharide affects stability of polysaccharide adhesion and influence properties of bacterial EPS. Moreover, the aliphatic and alcohol groups in polysaccharide had low activities to chlorine reactivity due to the saturated carbon ring structure.

4.1.5 Protein content of extracted EPS

Protein is the major component on EPS as same as polysaccharide. Protein analysis in this research was analyzed by BCA assay test kit (Cell signaling technology, microplate scale). Protein content of extracted EPS was measured by spectrophotometer via the absorbance at 540 nm wave range. Figure 4.2 illustrate the calibration curve for measuring the protein content. And the protein content of extracted EPS was shown in the table 4.8.

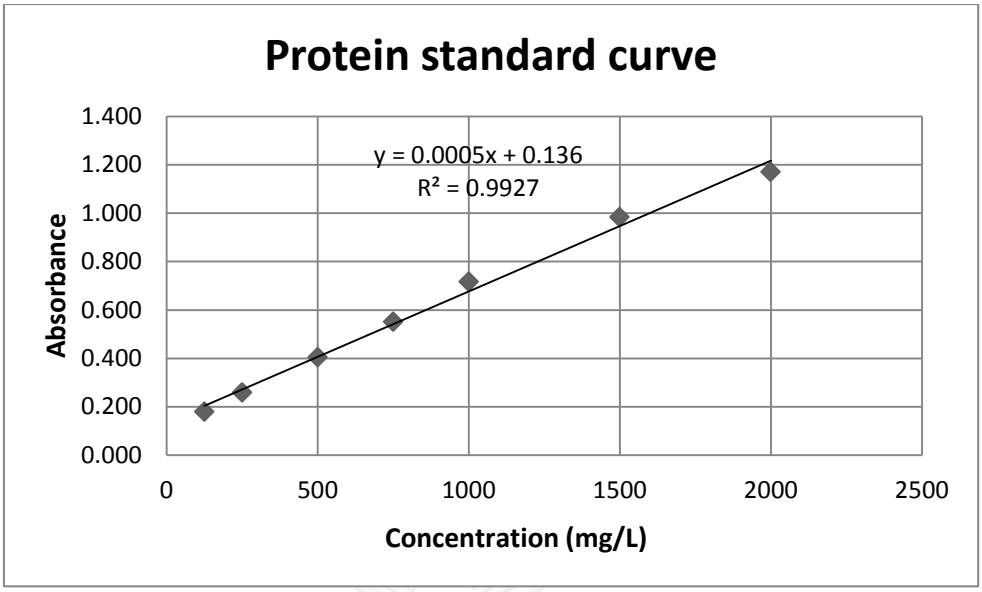


Figure 4.2 The calibration curve for measuring the protein content

Table 4.8 The protein content of extracted EPS

EPS Sample	Absorbance (Abs)	Concentration (mg/L)	% Weight of Protein / TVS of biofilm (%g / g TVS)	% Weight of Protein / TS of biofilm (%g / g TS)
	0.320	368	5.79	1.70
	0.312	352	5.54	1.63
	0.314	356	5.60	1.64
	Average	359	5.64	1.66

Protein concentration in extracted EPS solution was 359 mg/L. Or it can be calculated to be 5.64 % weight of protein / TVS of biofilm (%g / g TVS). It can be seen the content of protein in EPS was one time higher than saccharide content. Extracted EPS in this study had protein content higher than polysaccharide, similarly to the previous study. Wang (2011) reported that protein and polysaccharide component of EPS of *Pseudomonas putida*, which is commonly found in drinking water distribution system was 88.4% and 11.6% respectively.

From the study of Wang (2013), they found that in the same amount, the protein content in the EPS gave the overall DBPs yield higher than saccharide.

Moreover, protein, which is containing conjugate structure like Histidine and phenolic structures like Tyrosine were correlated to have high DBPs yield. Formation potential in previous study reported that Tyrosine has high CF yield, thus Tyrosine might be the significant precursor of THM in this study. HAAs formation potential might be based on Asparagine which has amine group on α-carbon. For HANs appeared that the formation could be attributed to the protein content in extracted EPS.

4.2 Disinfection by-products reduction by biological activity of biofilm

Drinking water distribution pipe or reservoir which is used for a long time has a chance for many culture of microbial growth and attach on the pipe surface. Free chlorine residue in drinking water can contact with the biofilm and form DBPs. However, the reduction of DBPs in the drinking water distribution pipeline might occur due to the abiotic reaction and biofilm activity.

Towards better understand of the fate and transport of the DBPs in drinking water distribution system this experiment were investigated the influence of biological activity on DBPs removal for each group of DBPs.

After quantifying biofilm, three concentration of DBPs (Chloroform (CF), Dichloroacetic acid (DCAA), Dichloroacetonitrile (DCAN) and 1,1 Dichloro-1-propanone (1,1DCP)) 100, 300 and 500 ppb was perform with 3 parameters of biofilm (control, active and inactive biofilm) to determine the mechanism of DBPs reduction for 24 hours.

The reduction of DBPs due to microbial activity was investigated and compared the result between the active and inactive biofilm. The main objective of this experiment is to clarify the effect of biological reduction of DBPs by biofilm.

4.2.1 Chloroform (CF) reduction experiment

Chloroform was selected to be the representative of THMs group for study the DBPs reduction by inactive and active biofilm, respectively. A 100, 300 and 500 ppb of chloroform were filled in the flask which had the fixed amount of active or inactive biofilm at 50 mg-C/L (under pH 7, ionic strength = 0.01 M). The mixtures were checked for 24 hours and then determine the CF concentration. The reduction capacities of chloroform were concluded via 3 normalized parameters (g-carbon, g-total solid and g- total inorganic solid) of biofilm and compared between of effect of active and inactive biofilm as shown in table 4.9.

Table 4.9 The reduction of CF by active and inactive biofilm**.**

	Control (µg/L)	CF Concentration Remain (µg/L)	µg CF Consume/g-C of biofilm	µg CF Consume/g TS of biofilm	µg CF Consume/g TFS of biofilm
Active Biofilm	110.8	108.3±1.4	2.1±1.1	0.2±0.1	0.3±0.2
	307.1	269.5±14.5	30.1±11.6	3.2±1.3	4.6±2.8
	464.2	404.9±16.5	47.4±13.2	5.1±1.4	7.2±2.0
Inactive Biofilm	110.8	110.4±0.5	0.3±0.4	0.03±0.03	0.04±0.1
	307.1	295.7±4.3	7.5±3.5	0.8±0.4	1.1±0.5
	464.2	440.8±7.2	18.2±5.8	2.0±0.6	2.8±0.9

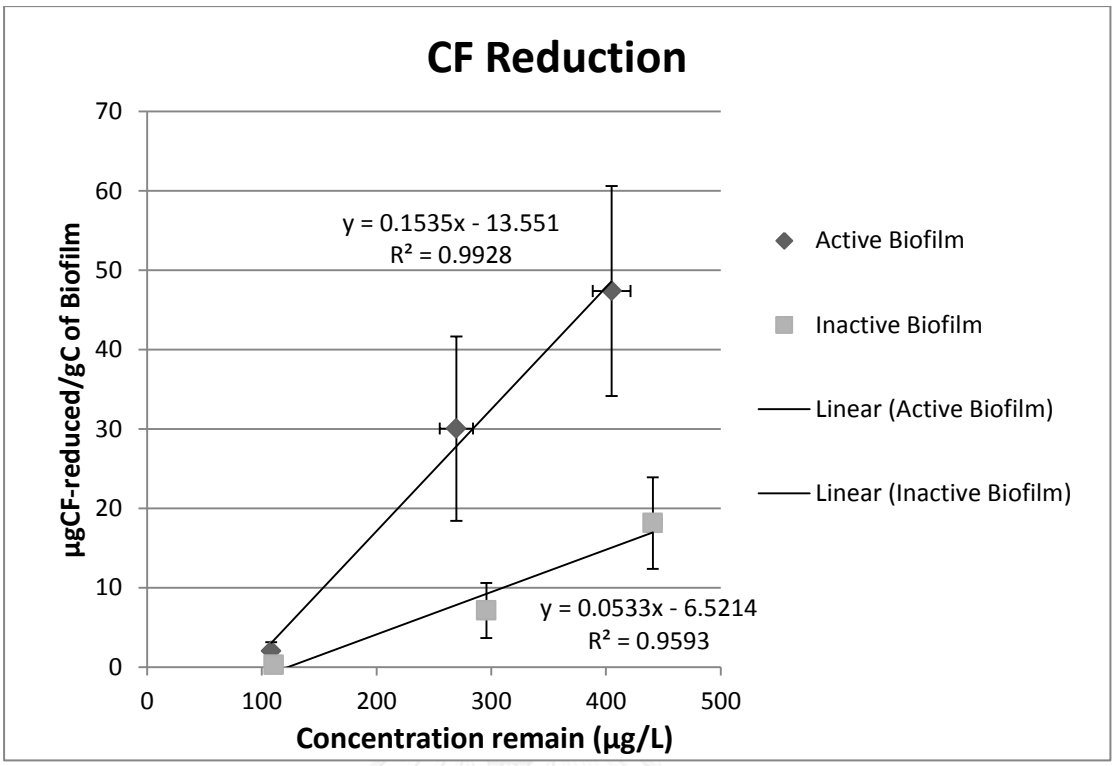


Figure 4.3 Reduction capacities of chloroform (CF) by active and inactive biofilm

After 24 hours of mixing, the amount of chloroform 2.1, 30.1 and 47.4 µg-chloroform/gram carbon of active biofilm were removed while the amount of reduced chloroform of inactive biofilm was 0.3, 7.5 and 18.6 µg chloroform per gram carbon of inactive biofilm. All of reduction capacities are related to the initial concentration of CF.

According to the result shown in table 4.9 and Figure 4.3, removal capacity of CF by active biofilm was clearly higher than the inactive biofilm. It indicated that there was an uptake mechanism by alive microorganism in active biofilm. And it can be also implied that CF can be biologically consumable or degraded by biofilm with attached on water reservoir or pipeline in water distribution system.

Moreover, at higher initial concentration of chloroform, the uptake of chloroform per gram carbon of biofilm tends to have higher concentration relatively. The relation presented both in active and inactive biofilm, so it might be caused from the abiotic reaction of biofilm. At higher concentration provided higher driving force for the reduction of chloroform.

However, THMs group is always found in water supply system and classified to be the main DBPs group (Wang). From these results, it can be suggested that some part of occurred THMs from chlorination process (disinfection process) can be removed by the presence of biofilm in distribution system. But, the THMs uptake efficiency should be compared with the THMs formation potential to identify the occurrence fate and transportation of THMs in the system again.

4.2.2 Dichloroacetic acid (DCAA) reduction experiment

DCAA was selected to be the representative of haloacetic acids group (HAAs). As same as in CF reduction experiment, initial concentration of DCAA at 100, 300 and 500 ppb was applied and pH at 7 and ionic strength at 0.01 M were controlled by phosphate buffer. 50 mg-C/L of active and inactive biofilm was employed and mixing time was 24 hours period to determine the DCAA concentration by GC-ECD.

The mixtures were shaken for 24 hours and then determine the DCAA concentration. The reduction capacities of DCAA were concluded via 3 normalized parameters (g-carbon, g-total solid and g- total inorganic solid) of biofilm and compared between of effect of active and inactive biofilm as shown in table 4.10.

Table 4.10 Reduction of DCAA by active and inactive biofilm.

	Control	DCAA Concentration Remain (µg/L)	µg DCAA Consume/g-C of biofilm	µg DCAA Consume /g TS	µg DCAA Consume/ g TFS of biofilm
Active Biofilm	101.2	84.9±2.8	11.8±2.3	1.3±0.2	1.8±0.3
	306.7	304.4±27.3	13.0±22.1	1.4±1.5	2.0±0.3
	578.6	529±33.6	45±26.9	4.9±2.9	7.0±4.1
Inactive Biofilm	129.8	103.7±3.2	14.2±8.7	1.5±0.9	2.2±1.3
	331.9	308.2±20.5	29.4±16.4	3.2±1.8	4.5±2.5
	577.3	538.3±43.9	31.1±35.2	3.3±3.8	4.7±5.3

After 24 hours of mixing, the amount of DCAA 11.8, 13.0 and 45.3 µg-DCAA/gram carbon of active biofilm were removed while the amount of consumed chloroform of inactive biofilm was 14.2, 29.4 and 16.4 µg-DCAA / gram carbon of inactive biofilm. All of reduction capacities are related to the initial concentration of DCAA.

The result obtained from table 4.10 and figure 4.4 show the reduction of active and inactive biofilm was not significantly different. This insignificant different indicated that there wasn't any DCAA uptake by biological activity. This means that DCAA might not be biologically consumable, so the reduction of DCAA by contacting with biofilm mainly occurred due to the abiotic reaction of the biofilm.

DCAA and CF abiotic removal by inactive biofilm at remaining concentration at 400 µg/L were 25 and 15 µg /g-carbon of biofilm, respectively. This can imply that DCAA can be attached on biofilm surface easier than CF, and this phenomenon might relate to hydrophilic characteristic of biofilm itself. However, further investigation might be needed to understand this abiotic mechanism.

Moreover, at higher initial concentration of DCAA, the uptake of DCAA per gram carbon of biofilm tend to have higher concentration as same as in chloroform case. This relation presented both in active and inactive biofilm, so it might be concluded that higher concentration provided higher driving force for the reduction of DCAA.

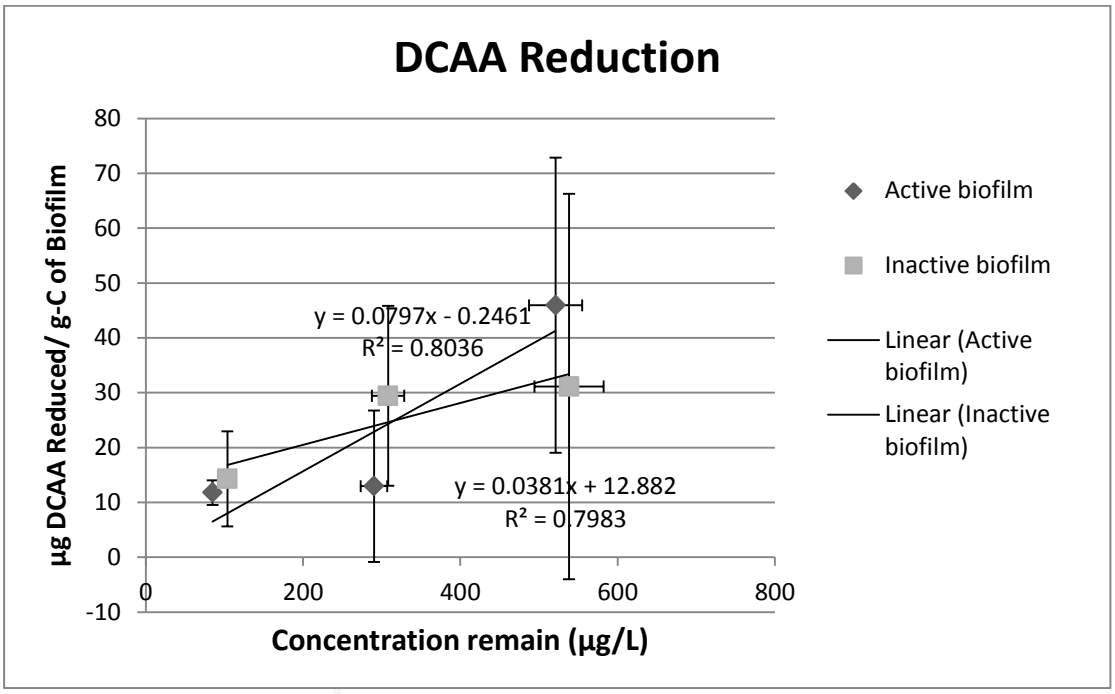


Figure 4.4 Reduction capacities of DCAA by active and inactive biofilm

4.2.3 Dichloroacetonitrile (DCAN) reduction experiment

DCAN was selected to be the representative of haloacetonitrile group (HANs). As same as in CF and DCAA reduction experiment, initial concentration of DCAN at 100, 300 and 500 ppb was applied and pH at 7 and ionic strength at 0.01 M were controlled by phosphate buffer. 50 mg-C/L of active and inactive biofilm was employed and mixing time was 24 hours period to determine the DCAN concentration by GC-ECD.

The mixtures were shaken for 24 hours and then determine the remained DCAN concentration. The reduction capacities of DCAN were concluded via 3 normalized parameters (g-carbon, g-total solid and g- total inorganic solid) of biofilm and compared between of effect of active and inactive biofilm as shown in table 4.11 and figure 4.5.

Table 4.11 Reduction capacities of DCAN by active and inactive biofilm.

	Control	DCAN Concentration Remain (µg/L)	µg DCAN Consume/g-C of biofilm	µg DCAN Consume/g-TS of biofilm	µg DCAN Consume/g-TFS of biofilm
Active Biofilm	92.08	20.8±1.0	57.0±0.8	6.1±0.1	8.7±0.1
	273.86	94.5±9.5	143.5±7.6	15.4±0.8	21.8±1.2
	465.65	182.8±12.3	226.4±9.8	24.3±1.1	34.3±1.5
Inactive Biofilm	99.64	98.3±0.9	1.1±0.7	0.1±0.1	0.2±0.1
	273.14	254.8±8.5	14.7±6.8	1.6±0.7	2.2±1.0
	430.73	390.7±10.0	32.1±8.0	3.4±0.8	4.9±1.2

After shaking for 24 hours was removed by active biofilm at 57, 143 and 226 µg-DCAN/gram carbon of active biofilm related to the initial DCAN concentration. In contrast, the less amount of DCAN can be removed by inactive biofilm at 1, 14 and 32 µg DCAN / gram carbon of inactive biofilm, respective to initial concentration. These results reveal that DCAN can be consumed by active biofilm easier that the previous CF and DCAA and it is also interesting that the presence of active biofilm in water distribution system (in pipe line and water reservoir) might help to reduce the concentration of DCAN (or HANs groups) that occurred by chlorination process.

According to the table 4.11, the maximum reduction of active and inactive at same initial DCAN concentration were approximately 226 and 32 µg DCAN per gram

carbon of biofilm, respectively. This considerably different of DCAN reduction between active and inactive biofilm indicated that DCAN was intensely uptaken by biological activity of biofilm. And DCAN is strongly biologically consumable than CF and DCAA.

Moreover, the DCAN removal by inactive biofilm at remaining concentration around 400 µg/L was approximately 32 µg-DCAN/g-carbon of biofilm, which is almost the same as what be found in case of DCAA and higher than CF.

As same as the previous experiment, at higher initial concentration of DCAN showed higher uptake of DCAN per gram carbon of biofilm and have clear relationship with initial concentration of DCAN (as same as CF and DCAA). This relation occurred both in active and inactive biofilm. Hence, it can confirm that the higher concentration might provide higher driving force for the reduction of DCAN.

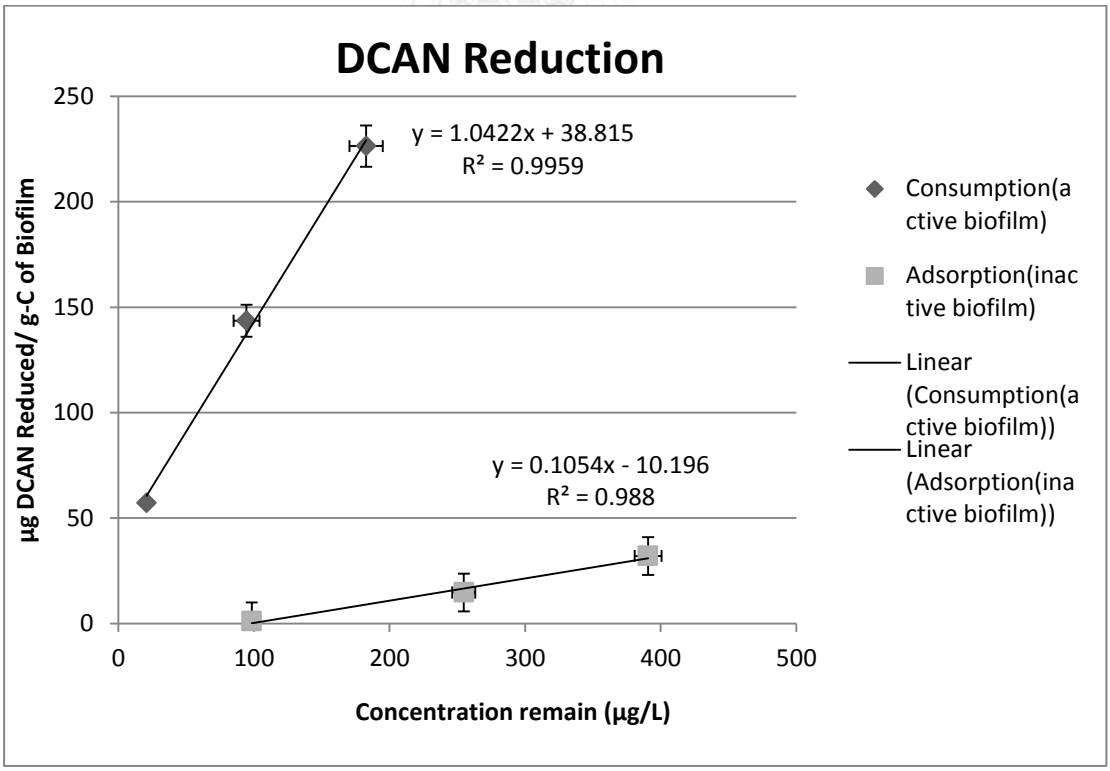


Figure 4.5 Reduction of DCAN by active and inactive biofilm

4.2.4 1,1-Dichloro-2-propanone (1,1-DCP) reduction experiment

1,1-DCP was selected to be the representative of haloketone group (HKs). As same as in CF, DCAA and DCAN reduction experiment, initial concentration of 1,1-DCP at 100, 300 and 500 ppb was applied and pH at 7 and ionic strength at 0.01 M were controlled by phosphate buffer. 50 mg-C/L of active and inactive biofilm was employed and mixing time was 24 hours period to determine the 1,1-DCP concentration by GC-ECD.

The mixtures were shaken for 24 hours and then determine the remained 1,1-DCP concentration. The reduction of DCAN were concluded via 3 normalized parameters (g-carbon, g-total solid and g- total inorganic solid) of biofilm and compared between of effect of active and inactive biofilm as shown in table 4.12 and figure 4.6.

Table 4.12 Reduction of 1,1 DCP by active and inactive biofilm.

	Control	1,1 DCP Concentration Remain (µg/L)	µg 1,1 DCP Consume/g-C of biofilm	µg 1,1 DCP Consume/g-TS of biofilm	µg 1,1 DCP Consume/g-TFS of biofilm
Active Biofilm	100.3	45.9±1.2	43.5±0.9	4.7±0.1	6.6±0.1
	297.2	174.2±8.8	98.5±6.5	10.6±0.7	14.9±1.0
	498.6	292.1±1.2	165.2±8.9	17.7±1.0	25.1±1.4
Inactive Biofilm	100.3	85.7±5.5	13.5±4.4	1.5±0.5	2.1±0.7
	297.2	280.4±4.9	14.2±3.9	1.5±0.4	2.2±0.6
	498.6	468.0±8.3	21.7±6.6	2.6±0.7	3.7±1.0

After mixing for 24 hours the amount of 1,1 DCP 43, 98 and 165 µg-1,1 DCP/gram carbon of active biofilm were reduced by active biofilm. In contrast, the less amount of DBPs decreasing due to abiotic reaction was found from inactive biofilm which was 13, 14 and 21 µg 1,1 DCP/ gram carbon of inactive biofilm. All obtained results had very good relation with 1,1 DCP initial concentration.

According to the table 4.12 and figure 4.6, the maximum 1,1 DCP reduction of active and inactive biofilm were approximately 226 and 32 µg per gram carbon of biofilm, respectively, at the same initial concentration. Similar to DCAN, this considerably difference of 1,1 DCP removal efficiencies between active and inactive biofilm indicated that 1,1 DCP was substantially uptaken by biological activity; therefore, it might be concluded that 1,1 DCP is also biologically consumable like DCAN. However, DCAN performed the highest uptake capacity comparing with the other three DBPs group.

As same as the previous experiment, 1,1 DCP uptake efficiency trend to be higher together with the increasing of initial concentration. And this relation presented both in active and inactive biofilm. The higher concentration also provided higher driving force for the reduction of 1,1 DCP.

As we have discussed in DCAN case, the presence of active biofilm strongly relate to the reduction of HANs, HKs and THMs, respectively. This mechanism might affect the concentration of these three DBP groups in tap water. However, the occurrence of these DBP groups also relate to the formation potential of each groups and both of reduction by biological reduction and formation potential should be taken in account to evaluate the risk of DBPs in water distribution system.

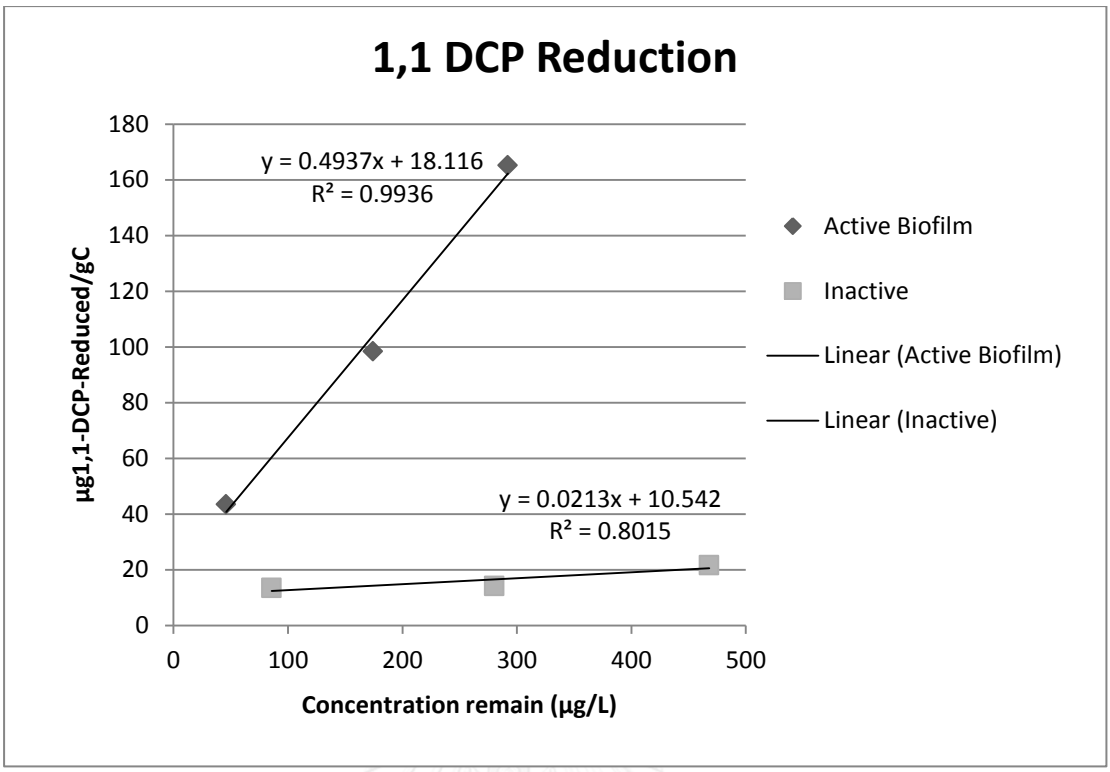


Figure 4.6 Reduction capacities of 1,1 DCP by active and inactive biofilm

4.2.5 Effect of molecular structure of DBPs on DBPs reduction

Figure 4.7 illustrate the comparison of reduction of studied four DBPs on active biofilm. From figure 4.8, DCAN was the most biologically consumable, therefore it might cause the reduction of the DCAN formation in part 4.3.2. There was no significant different between DCAA and CF with the low reduction by biofilm.

It can be seen that DCAN and 1,1 DCP had higher reduction than CF and DCAA. However, comparing the obtained data and physico-chemical properties of these four DBPs showed that reduction capacities do not have any relationship between molecular size (or water solubility and halogenic atom) and reduction. (Water solubility of CF, DCAA, DCAN and 1,1 DCP were 8.09 g/L, 86.3 g/L, 25 g/L and 0.748 g/L, respectively.)

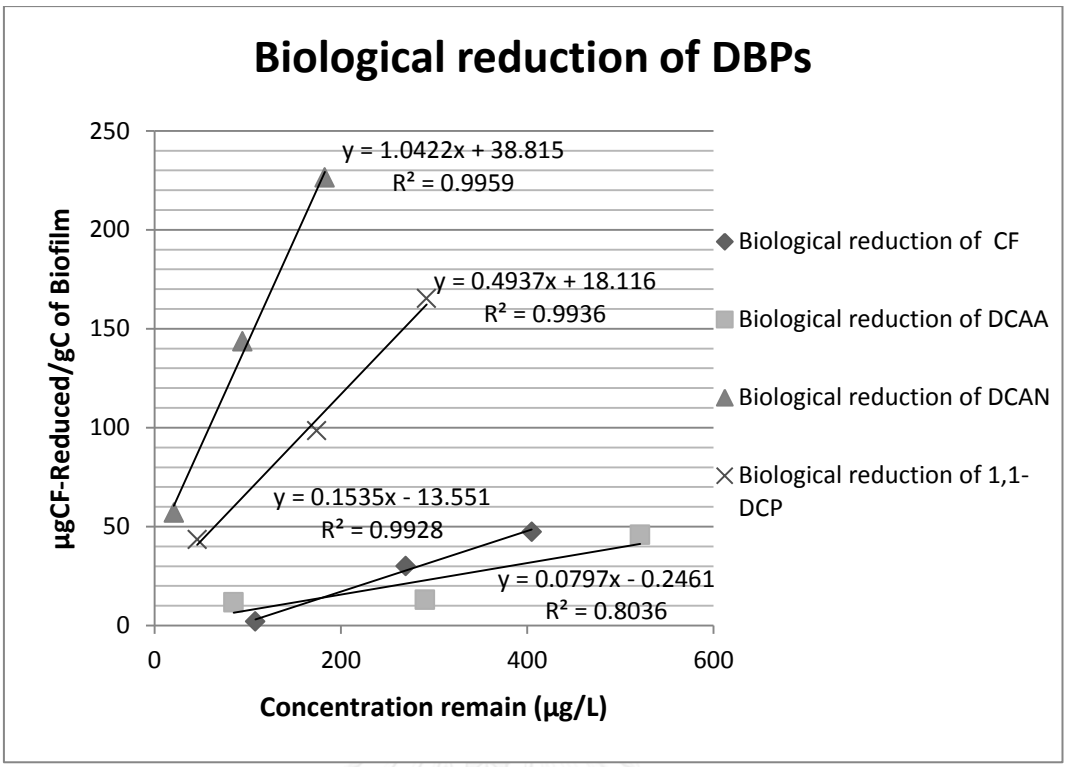


Figure 4.7 Biological reduction of CF, DCAA, DCAN and 1,1 DCP by active biofilm

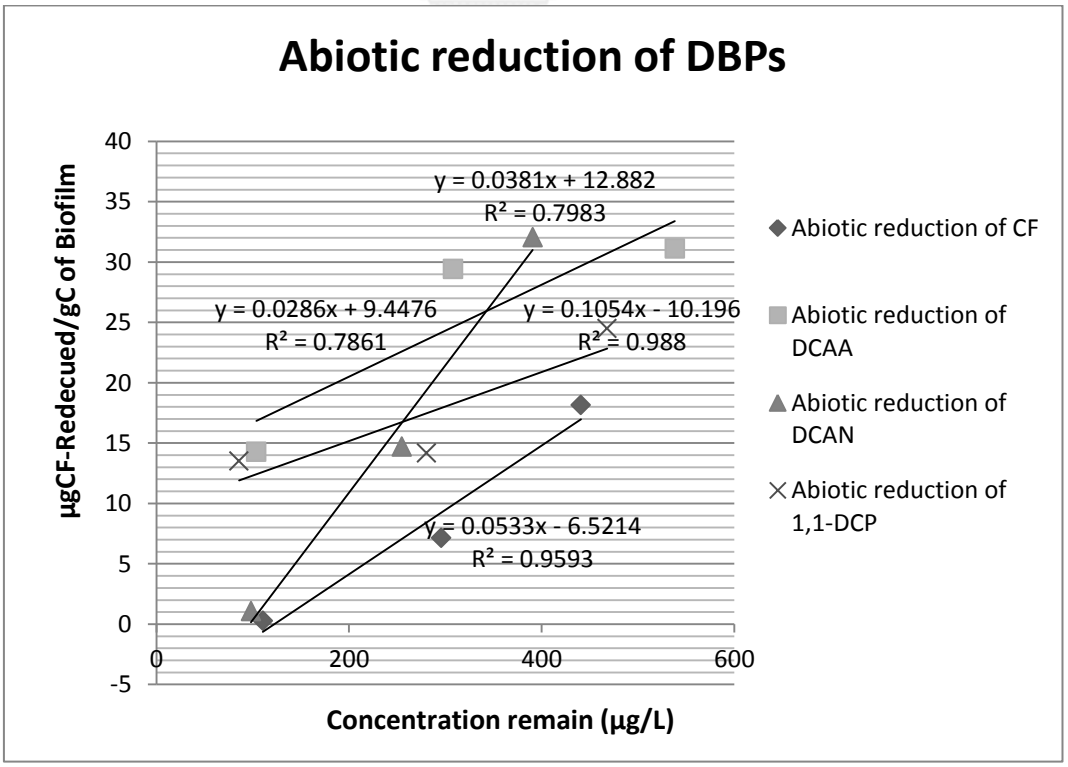


Figure 4.8 Abiotic reduction of CF, DCAA, DCAN and 1,1 DCP by inactive biofilm

Figure 4.8 show the comparison of abiotic reduction of four DBPs on the surface of inactive biofilm. It was found that CF seems to have the lowest abiotic reduction capacity, while the DCAA tend to have highest abiotic reduction capacity. And DCAN and 1,1DCP had abiotic reduction capacities in the mid range of all target DBPs. As same as the obtained results of active biofilm (Figure 4.7), it might be concluded that there is no relationship between physico-chemical properties of the DBPs and physical abiotic reduction capacity on inactive biofilm.

4.3 Disinfection by-products formation potential

According to the previous literature survey, the presence of biofilm in drinking water distribution system is proved to be one of the DBPs organic compound precursors which can increase the DBPs concentration in tap water. Bacterial cultures which are attached on the pipe or reservoir surface can excrete EPS and form biofilm structure (More et al., 2014), therefore the precursor of DBPs might be occurred from the reaction between biofilm and free chlorine residue which maintained in water distribution system until the water reached consumer's end tap.

For better understanding of DBPs formation potential (DBPs-FP) in the distribution pipe line and reservoir, this experiment was conducted for determination of maximum DBPs formation potential. The experiment was performed 10 mg-C/L either biofilm or EPS with head space-free and excess free chlorine for 7 days at pH 7, 30℃ and 0.01M ionic strength to reach the maximum formation potential. 7 days incubation time was selected in this study in order to investigate the maximum formation potential for both biofilm and EPS.

After 7 days, each sample still had free chlorine residue and the DBPs formation potential was analyzed by GC-ECD and the DBPs yield of biofilm and extracted EPS was shown in table 4.13 and figure 4.9-4.13.

4.3.1 Chloroform (CF) formation potential

From figure 4.9, CF formation potential of both biofilm and EPS is quite same at 14.5 and 14.4 µg /mg-C, respectively. The ratio of EPS reported from literature (Bakke et al., 2001) can be up to 30 %w/w, and in this study the ratio of EPS was around 14.89 %w/w. Hence, if we compare the CF formation potential (base on the same carbon content), it seems to be that the cellular carbon might have higher potential to form CF than the carbon source from extract EPS.

Table 4.13 DBPs formation potential of biofilm and EPS at 7 days incubation and pH 7

Group of DBPs	Species	Biofilm (µg /mg-C)	SD	EPS (µg /mg-C)	SD
Trihalomethane (THM)	CF	14.5	1.5	14.4	2.5
Haloacetic acids (HAAs)	MCAA	3.5	9.5	4.1	0.6
	DCAA	6.3	1.8	9.6	1.1
	TCAA	6.7	1.2	10.6	3.1
Haloacetonitriles (HANs)	MCAN	2.5	0.1	4.3	0.1
	DCAN	0.8	0.2	1.0	0.1
	TCAN	ND	-	0.4	0.04
Haloketones (HKs)	1,1 DCP	9.8	0.1	2.7	0.1
	1,1,1 TCA	2.5	0.1	0.4	0.03

ND : LOD of TCAN = 0.10 µg/L

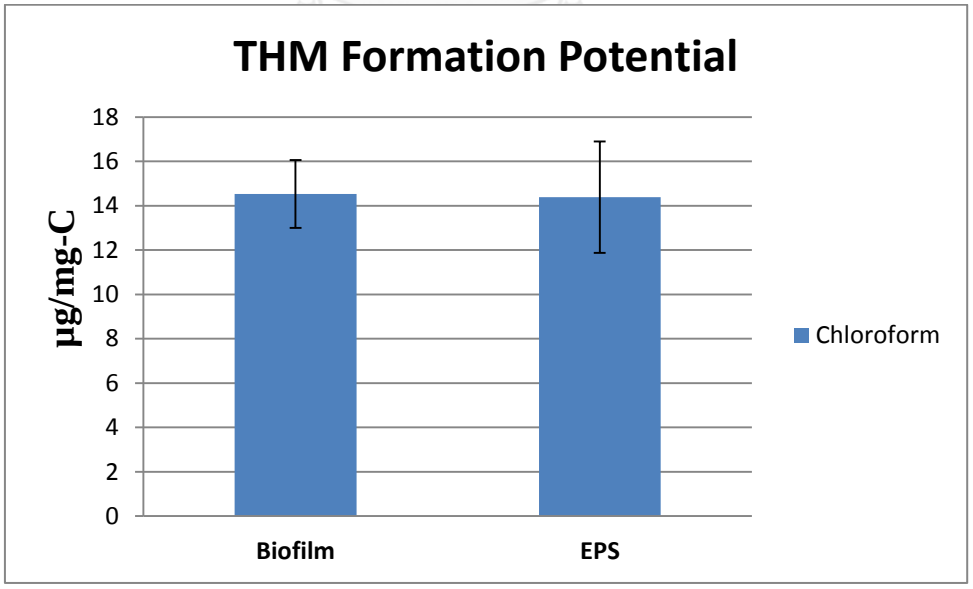


Figure 4.9 CF formation potential of biofilm and extracted EPS (7 days incubation)

4.3.2 Haloacetic acids (HAAs) formation potential

Figure 4.10 illustrate the HAAs formation potential and emphasized just only chlorinated HAAs (MCAA, DCAA and TCAA). Form obtained data, TCAA was found to have the highest formation potential approximately 6.7 and 10.7 µg /mg-C for biofilm and extracted EPS, respectively. And in both cases (biofilm and EPS), DCAA and MCAA formation potential were detected slightly lower than TCAA, respectively.

As mentioned above, the chlorine residue concentration was controlled to be enough for 7 day reaction. It means that the reaction had enough halogenic species to react with carbon source in the reactor. Hence, it might be suggested that TCAA which has highest halogenic atom in molecular structure should be the main product for this 7 day incubation with excess chlorine residue.

Comparing with the carbon source from biofilm and EPS, it was clearly found that all HAAs formation potentials of the carbon source from EPS were higher than that from biofilm. It might relate to the ratio between cellular carbon and EPS carbon in the reaction. Biofilm's sample, which had lower HAAs formation potential, had mixed carbon sources from cellular and EPS carbon source (at least 14.89 % w/w). While, the EPS's sample, which had 100% of carbon source from EPS, can give higher HAAs formation potential. Hence, it can be suggested that HAAs formation potential has closer relation to the carbon source from EPS than that from cellular source.

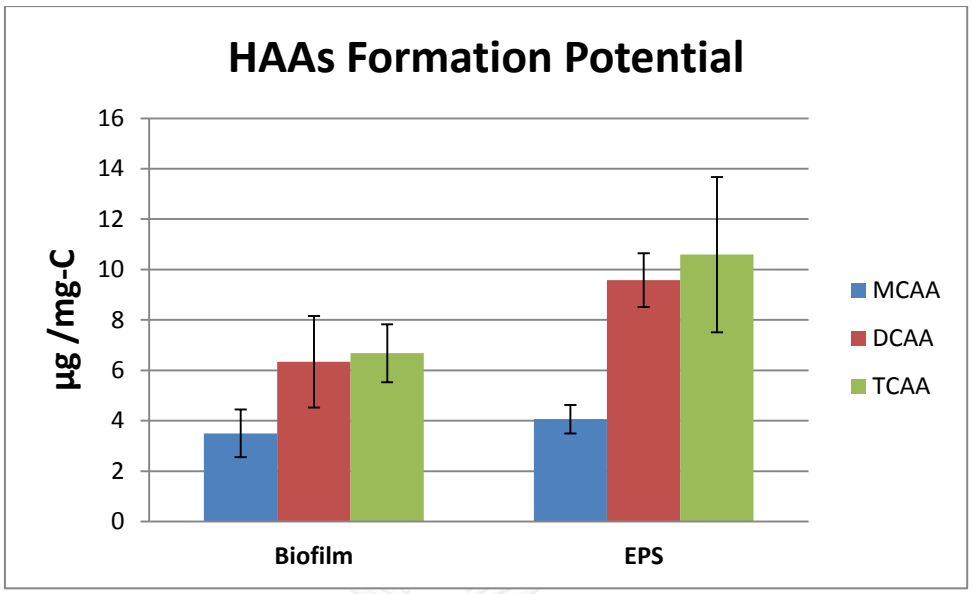


Figure 4.10 HAAs formation potential of biofilm and extracted EPS (7 days incubation)

4.3.3 Haloacetonitriles (HANs) formation potential

From figure 4.11, it can be seen that HANs formation potential of EPS was higher that biofilm. And MCAN showed highest formation potential for both of biofilm and EPS (2.5 and 4.3 µg /mg-C, respectively). As same as HAAs, organic source from EPS (carbon and nitrogen) seem to be the main precursor for HANs comparing with organic source from biofilm. However, obtained HANs formation potential was lowest comparing with other three DBPs formation potentials. This might be caused by the low protein content of EPS (around 5.6 % w/ w of TVS) which supposed to be the main nitrogen source for nitrogenous DBPs such as HANs.

Moreover, formation potential of MCAN in both case were higher that DCAN and TCAN. It can be supposed that the reaction to form HANs was limited and quite hard to occur comparing with THMs, HAAs and HKs. Comparing between nitrogenous DBPs and carbonaceous DBPs formation potential, it can be concluded

that carbonaceous DBPs had higher formation potential and had a trend to produce higher halogenic DBPs.

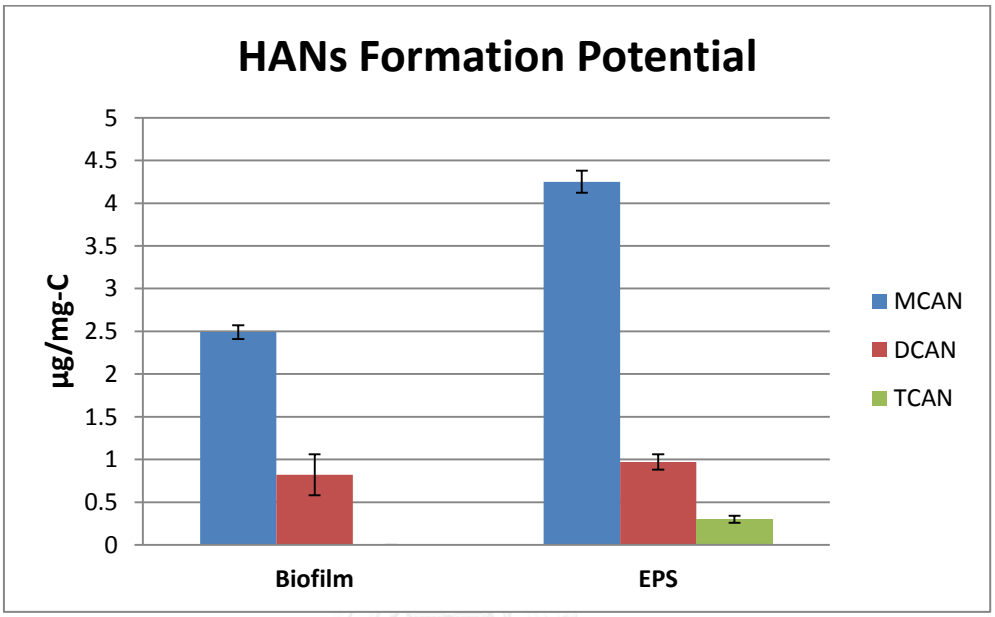


Figure 4.11 HANs formation potential of biofilm and extracted EPS (7 days incubation)

4.3.4 Haloketones (HKs) formation potential

Figure 4.12 illustrated the HKs formation potential of both biofilm and EPS. Lower haloginic atom of 1,1 DCP (9.8 and 2.7 µg /mg-C for biofilm and EPS, respectively) had higher formation potential than 1,1,1 TCA (2.4 and 0.4 µg /mg-C for biofilm and EPS, respectively). However, carbon source from biofilm presented higher overall HKs formation potential than that form EPS. Hence, it can imply that cellular carbon source of biofilm might be the main carbon source for HKs formation potential.

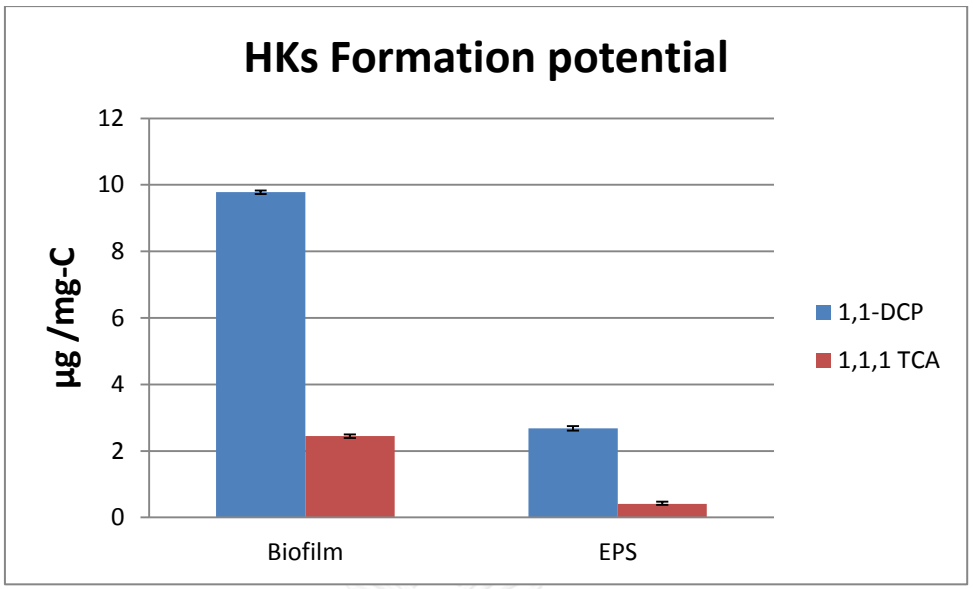


Figure 4.12 HKs formation potential of biofilm and extracted EPS (7 days incubation)

4.3.5 Comparison of DBPs formation potential

Figure 4.13 showed the overall DBPs formation potential form both biofilm and EPS. The obtained results showed that CF from THMs group had the highest yield for both in biofilm and extracted EPS, approximately 14 µg /mg-C following with HAAs and HKs formation potential. And HANs showed the lowest formation potential compared with these applied four groups of DBPs.

Comparison with Wang (2008), they reported that the highest yield of DBPs formation potential from extracted EPS was HAA_3 (HAA_3 is summation MCAA, DCAA and TCAA) approximately 60 µg /g-C and the yield of CF was still high when compare with other DBPs (approximately 40 µg /g-C). From the study of Hong et al. (2009), they found that the amino acid in aliphatic group had low yield of CF, but the yield of aromatic group, such as Tyrosine and Phenylalanine was higher. Tyrosine was reported that it might be one of the main precursors of THMs in biofilm. Phenolic structure such as Tryptophan was also reported as the main precursor of THMs.

For HAAs yield, the highest yield of HAAs was TCAA and DCAA with insignificant different and the yield was approximately 6 and 10 µg /mg-C for biofilm and extracted EPS respectively. MCAA was found less than a half of DCAA and TCAA. In contrast, Wang (1013) found that DCAA yield of extracted EPS was the main species formed for all amino acid group except the group that has aromatic functional. Aromatic group, such as Tyrosine produced higher TCAA yield than DCAA, therefore the collected biofilm in this study might consist of high aromatic functional protein. Moreover, the study found that Asparagine, Tyrosine and Histidine were the main precursor of HAAs and especially asparagine was the crucial precursor for the DCAA formation.

HANs was only one group that could be attributed mainly to protein, since the structure containing organic nitrogen is precursor for nitrogenous DBPs. For HANs formation potential, MCAN was found as the dominant species with much higher than other two species at 2.5 and 4.5 µg /mg-C for DBPs and extracted EPS respectively. In contrast, Wang (2013) reported that the major specie of HAN that he was found was DCAN. This different might relate to the composition of protein content in EPS. According to the study of Wang (2013), the main precursors of HANs formation was Asparatic acid and Asparagine.

For HKs, the highest yield of HKs was 1,1 DCP approximately 10 and 2.6 µg /mg-C from biofilm and extracted EPS, respectively. In contrast, Wang (2013) reported that 1,1 DCP was found in the low concentration.

In conclusion, overall comparison of DBPs formation potential of biofilm was CF >TCAA > 1,1 DCP > DCAA > MCAA > MCAN > DCAN >1,1,1TCA > TCAN, and DBPs formation potential of extracted EPS was CF > TCAA > DCAA > MCAN > MCAA > 1,1DCP > DCAN > 1,1,1TCA >TCAN. However, comparing with the previous literature which mostly done by using pure culture and surrogate protein and organic compounds, it was found that the sequences for DBPs formation potential were different from previous studies. That might be caused by the carbon and nitrogen precursors from biofilm and EPS used in this study were collected from real water reservoir that have a lot of uncontrolled contaminants.

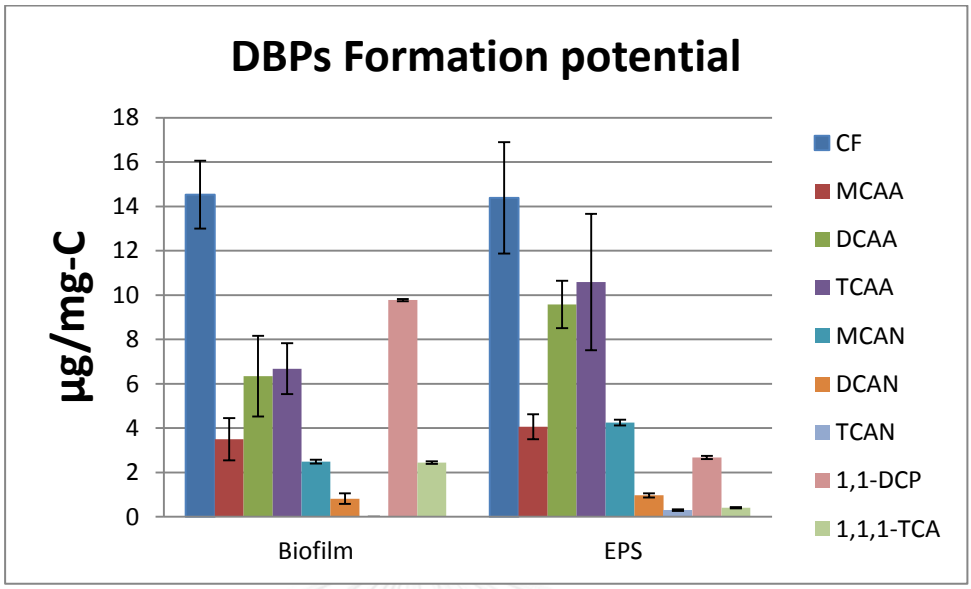


Figure 4.13 DBPs formation potential of biofilm and extracted EPS (7 days incubation)

CHAPTER 5
CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The main objective of this study is to investigate the DBPs formation potential of biofilm in water distribution system and to investigate the effect of biofilm on reduction of DBPs in water distribution system for better understanding of fate and transport of DBPs that might be occurred by biofilm in drinking water distribution system. The biofilm of this study was obtained from drinking water reservoir.

To investigate the reduction of DBPs biofilm and DBPs was added and agitated for 24 hours. This part was separated biofilm into 2 parameters active and inactive biofilm for comparison the reduction by microbial activity and abiotic reaction of biofilm. The result of reduction and abiotic control test shown that DCAN is the most biological consumable followed by 1,1 DCP, therefore it might cause the reduction of DCAN and 1,1 DCP concentration in drinking water distribution system. In contrast, CF and DCAA were slightly consumed by physical attachment on biofilm surface.

According to investigation of DBPs formation potential, biofilm and extracted EPS were chlorinated for 7 days to find the maximum formation potential of each DBPs species. After 7 days every sample still had free chlorine residue, therefore the maximum formation potential of all sample were reached. Chloroform yield was the highest both in biofilm and extracted EPS. Cellular carbon might have higher potential to form chloroform than the carbon source from extract EPS. HAAs formation potential has closer relation to the carbon source from EPS than that from cellular source. And cellular carbon source of biofilm might be the main carbon source for HKs formation potential. Carbonaceous DBPs had higher formation potential and had a trend to produce higher halogenic DBPs. For overall comparison of DBPs formation potential of biofilm and EPS is CF > TCAA > 1,1 DCP > DCAA > MCAA > MCAN > DCAN > 1,1,1TCA > TCAN and CF > TCAA > DCAA > MCAN > MCAA > 1,1DCP > DCAN > 1,1,1TCA > TCAN respectively.

5.2 Engineering significance

DBPs formation potential of biofilm as well as DBPs reduction by biological activity were investigated towards a better understanding the role of biofilm on the increasing and decreasing of DBPs in drinking water distribution system. DBPs can be generated by reaction between biofilm and free chlorine in distribution system. That obtained information can be applied for the development of the type of end tap unit to eliminate the DBPs, which is the precursor of human cancer. The end tap unit should be able to eliminate DBPs without generate or release any toxic substances into the tap water. This obtained information can be used for selecting the adsorbents (which have appropriate functional group, capacity and selectivity) for designing unit operation.

5.3 Recommendation and future work

As the result, the reduction of DBPs (THM, HAAs, HANs, and HKs) by biological activity and abiotic reduction were investigated just only the representative of each groups. However, furthers studies should investigate the mechanism of biological of the other DBPs for better understanding of DBPs fate and transport. Moreover, the combine unit process for eliminating DBPs in drinking water is the interesting alternative.

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APPENDIX

APPENDIX A

GC Chromatogram of THM, HAAs, HANs and HKs

Table A-1 THM HANs and HKs determination by Gas Chromatograph followed the modified EPA Method 551.1 (VFX, GC/ECD)

Retention time (min)	CF	3.32	3.01	3.94	4.04	6.89
HANs	2.78	MCAN	TCAN	1,1 DCP	DCAN	1,1,1 TCA
Detection Limit	0.05-0.10 µg L^{-1} or ppb					

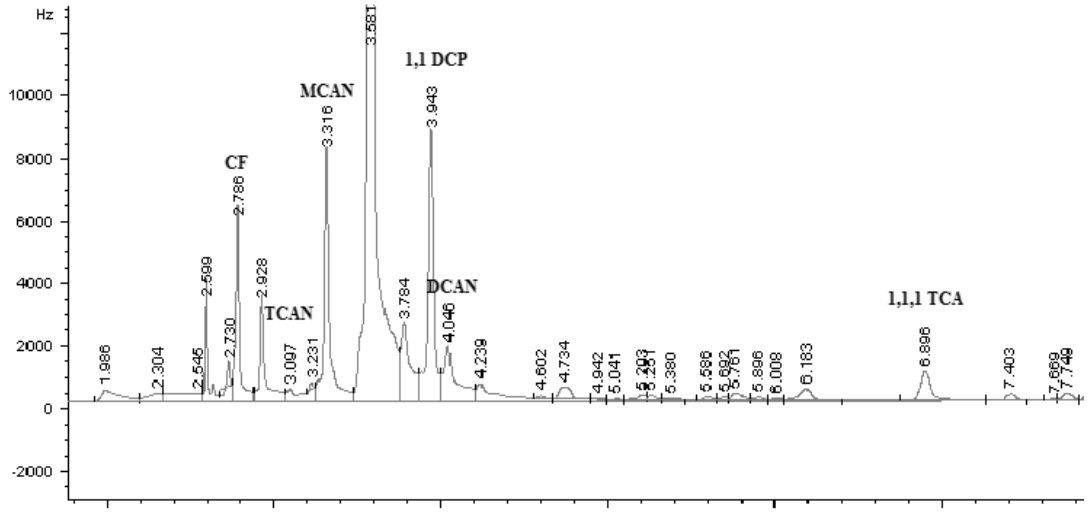
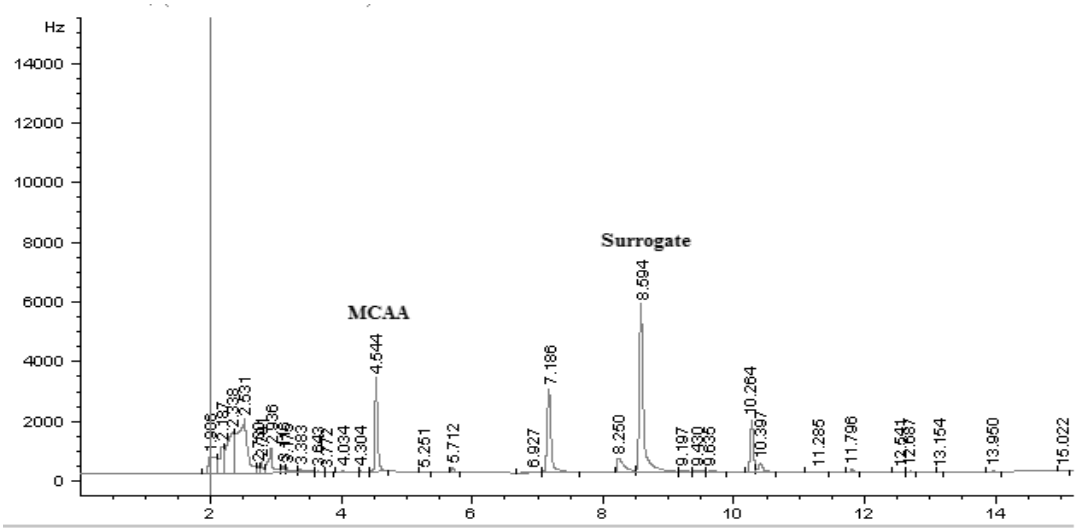
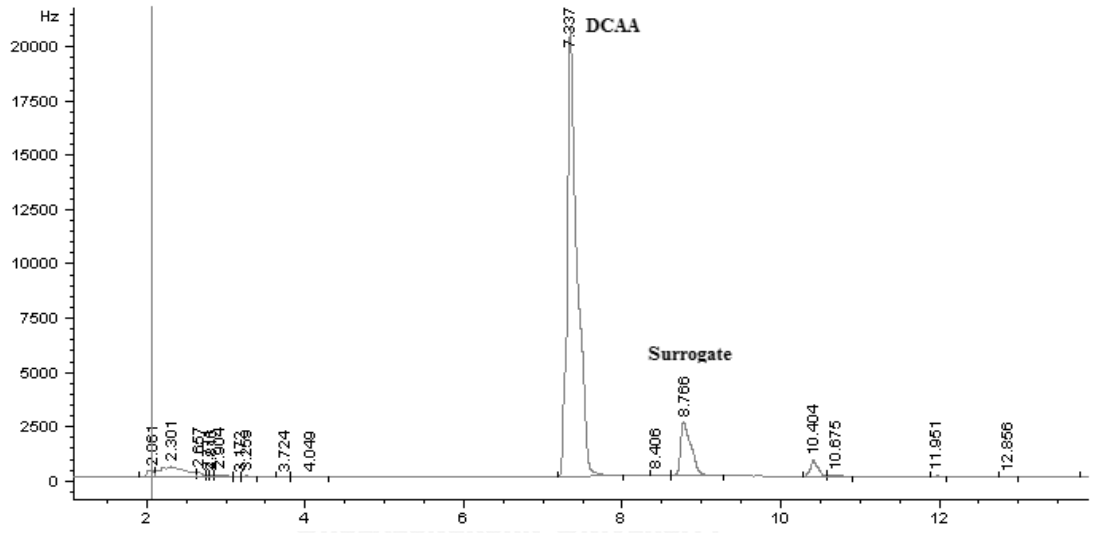
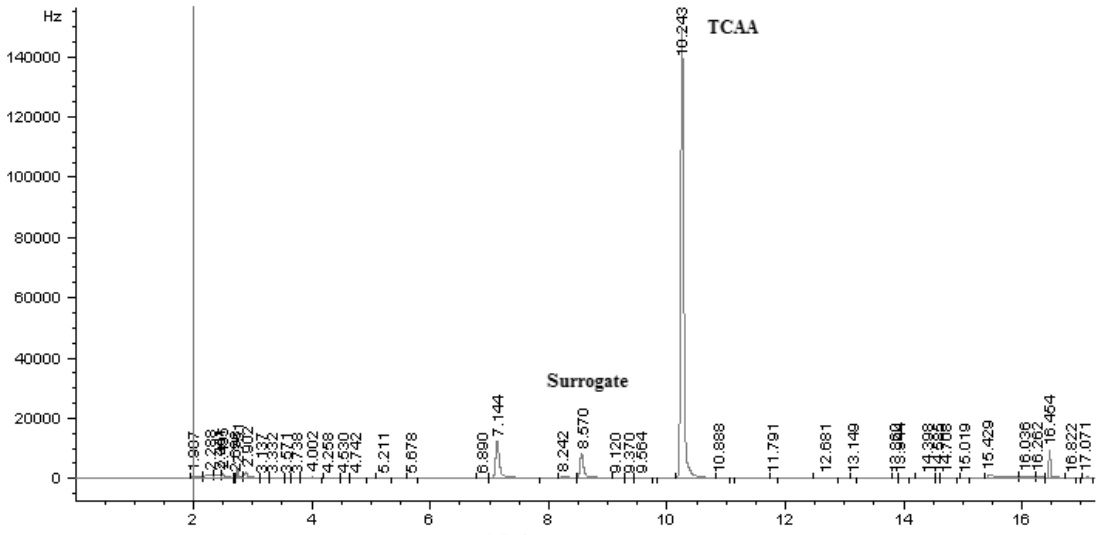


Table A-2 HAAs and Standard Surrogate determination by Gas Chromatograph followed the modified EPA Method 551.1 (VFX, GC/ECD)

Retention time (min)	4.54	7.17	10.26	8.59
HANs	MCAA	DCAA	TCAA	Surrogate
Detection Limit	0.05-0.5 µg L^{-1} or ppb			


Hz
MCAA
Surrogate
4.544
8.594
7.186
10.264
4.304
4.034
5.251
5.712
6.927
8.250
9.197
10.397
11.285
11.796
13.154
13.950
15.022


Hz
DCAA
Surrogate
7.337
8.766
2.061
2.301
3.724
4.049
8.406
10.404
10.675
11.951
12.856



APPENDIX B

Data for the investigation of DBPs Reduction

Table B-1 CF Data information for Reduction and investigation

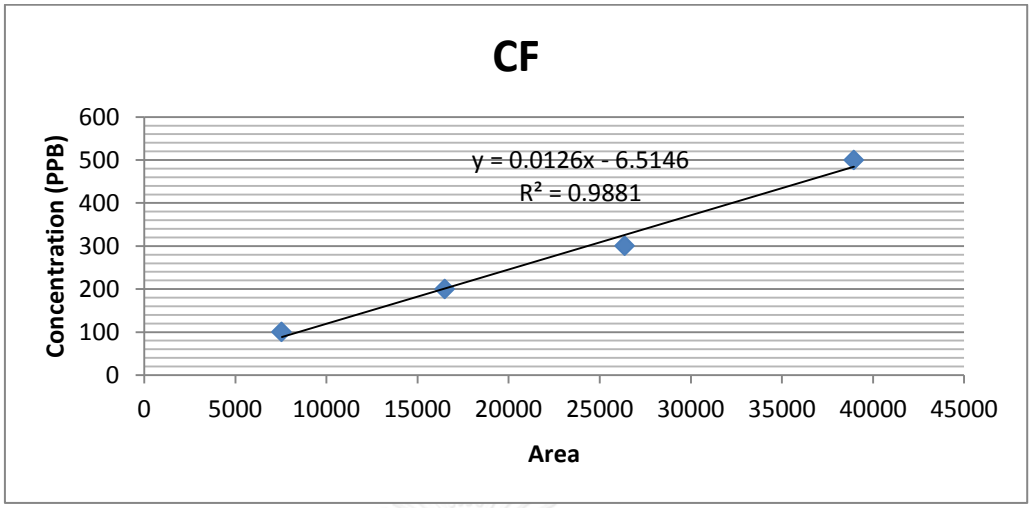


Figure B-1-1 CF standard curve

Reduction Test				
	Area	Concentration	mean	SD conc
BCF 100	9394.7	111.86		
BCF 100	9231.2	109.79	110.82857	1.456711
BCF 300	25011	308.62		
BCF 300	24769.7	305.58	307.10381	2.149873
BCF 500	38813.4	482.53		
BCF 500	35901.7	445.84	464.19053	25.94192

	Area	Concentration	Mean Conc	SD conc
ACF 100	9137.4	108.61		
ACF 100	9199.7	109.40	108.27	1.35
ACF 100	8991.4	106.77		
ACF300	20587.9	252.89		
ACF300	22424.7	276.03	269.53	14.52
ACF300	22712.6	279.66		
ACF500	33182.1	411.57		
ACF500	31162.1	386.12	404.94	16.53
ACF500	33621.3	417.11		
DCF100	9257.5	110.12		
DCF100	mistake	-	110.47	0.48
DFC100	9311.2	110.80		
DCF300	24199.1	298.39		
DCF300	23586.2	290.67	295.67	4.33
DCF300	24163.4	297.94		
DCF500	34992.1	434.38		
DCF500	35382.1	439.29	440.77	7.23
DCF500	36121.3	448.61		

B1,1 DCP = Control of 1,1 DCP

A1,1 DCP = Active biofilm with 1,1 DCP

D1,1 DCP = Inctive biofilm with 1,1 DCP

Table B-2 DCAA Data information for Reduction investigation

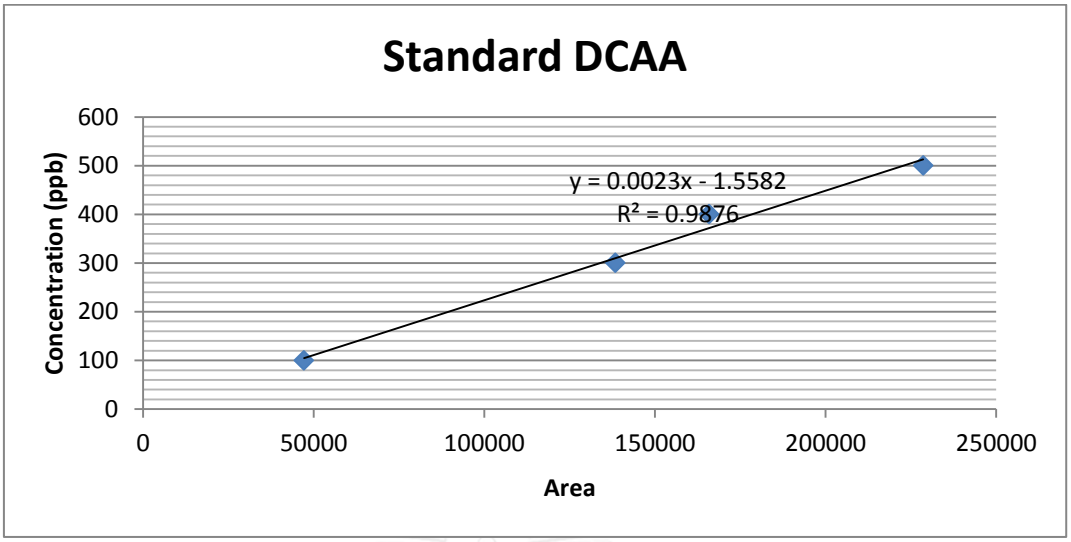


Figure B-2-1 DCAA standard curve

Standard Surrogate

surr conc (ppb)	RT	Area	DF	Area*DF	RF
20	8.776	10801.4	1	10801.4	0.001852
30	8.759	13637.1	1	13637.1	0.0022
40	8.757	23129.5	1	23129.5	0.001729
50	8.766	25104	1	25104	0.001992
60	8.767	30762	1	30762	0.00195
				avg	0.001945

Standard DCAA

DCAA (ppb)	Area*RF (ppb)	CF	DCAA area*DF*CF	% Recovery
100	48.02	1.04	38945.69	96.04
300	49.16	1.02	119199.4	98.32
400	49.49	1.01	153388.4	98.97
500	46.78	1.07	202942.2	93.57

Reduction Test		RF of surrogate = 0.001945		
	Area*DF*CF	Concentration	%recovery	SD conc
BDCAA 100	46875.65	101.24	107.26	
BDCAA 300	136987	306.72	93.65	1.457
BDCAA 500	218451.6	578.61	85.259	

	Area*DF	Concentration	Mean Conc remain	SD conc	%recovery
ADCAA100	40344.85	86.35			104.18
ADCAA100	38289.28	81.67			107.67
ADCAA100	40521.86	86.76	84.92	1.35	104.04
ADCAA300	20587.9	302.01			96.76
ADCAA300	22424.7	332.84			85.14
ADCAA300	22712.6	278.34	304.40	14.52	105.75
ADCAA500	33182.1	559.63			93.03
ADCAA500	31162.1	506.64			102.46
ADCAA500	33621.3	497.29	529.12	16.53	104.92
DDCAA100	43688.89	110.13			89.48
DDCAA100	mistake	-			91.75
DDCAA100	46879.44	110.81	110.47	0.48	78.89
DDCAA300	130991.5	298.39			85.3+
DDCAA300	116764	290.67			77.56
DDCAA300	116433	297.94	295.67	4.33	75.99
DDCAA500	227298.1	434.39			87.07
DDCAA500	132597.5	439.30			50.03
DDCAA500	202104.2	448.61	440.77	7.23	80.05

RF = Respond factor

DF = Dilute factor

BDCAA = Control of DCAA

ADCAA = Active biofilm with DCAA

DDCAA = Inactive biofilm with DCAA

Table B-3 DCAN Data information for Reduction investigation

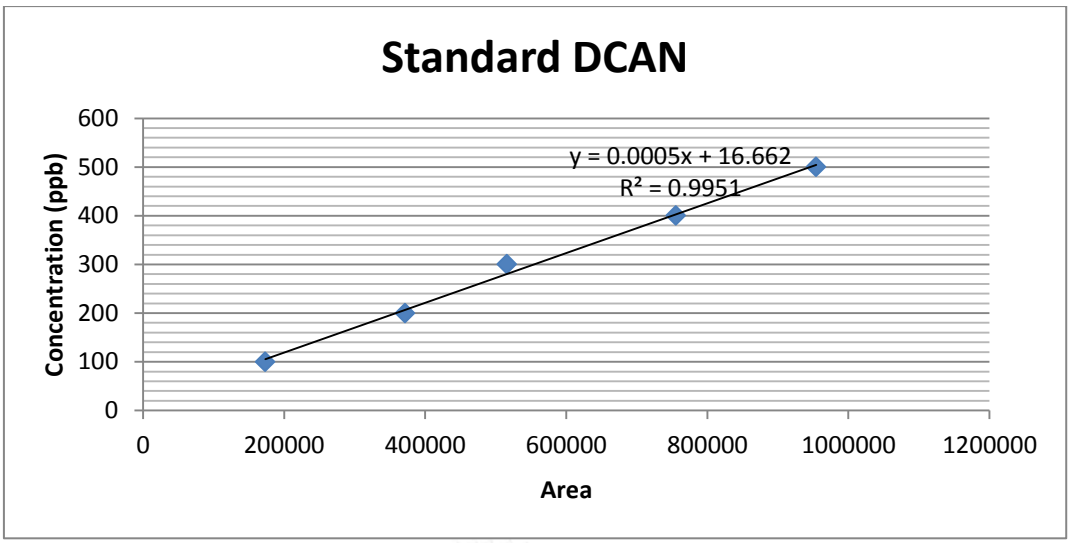


Figure B-3-1 DCAN standard curve for ADCAN

Control for active DCAN

Reduction Test		
Control for ADCAN	Area	Concentration
BDCAN 100	149435	92.07894
BDCAN 300	503134.6	273.8642
BDCAN 500	876292.4	465.6501

	Area	Concentration	Mean Conc remain	SD conc
ADCAN100	10751.4	20.80		
ADCAN100	8709.1	19.75		
ADCAN100	12621.1	21.76	20.77	1.01
ADCAN300	135906.9	85.13		
ADCAN300	160863	97.95		
ADCAN300	172192.9	103.78	95.62	9.54
ADCAN500	353342.8	196.88		
ADCAN500	312456.7	175.86		
ADCAN500	311415.1	175.33	182.69	12.29

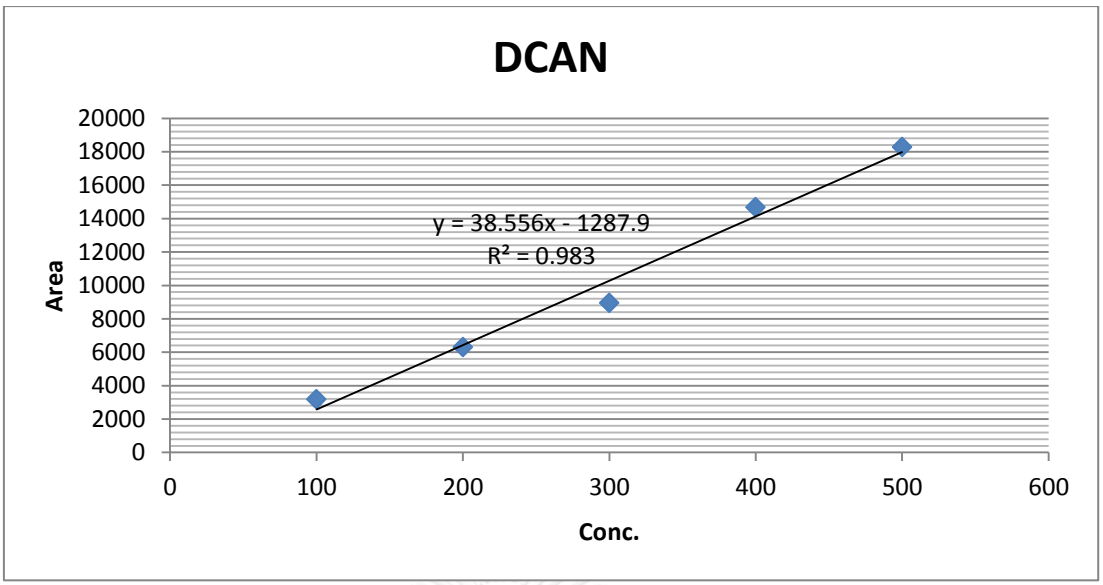


Figure B-3-2 DCAN standard curve for DDCAN

Control of DDCAN

Reduction Test		
Control for DDCAN	Area	Concentration
BDCAN 100	2553.8	98.84
BDCAN 300	9243.1	261.23
BDCAN 500	15319.7	430.74

	Area	Concentration	Mean Conc remain	SD conc
DDCAN100	2464	97.31		
DDCAN100	2518	98.71		
DDCAN100	2523.1	98.84	98.29	0.85
DDCAN300	8783.6	261.22		
DDCAN300	8906	264.39		
DDCAN300	8285.4	248.30	257.97	8.53
DDCAN500	14178.9	401.15		
DDCAN500	13734.7	389.63		
DDCAN500	13411.6	381.25	390.68	9.99

BCF = Control of CF

ACF = Active biofilm with CF

DCF = Inactive biofilm with CF

Table B-3 1,1-DCP Data information for Reduction investigation

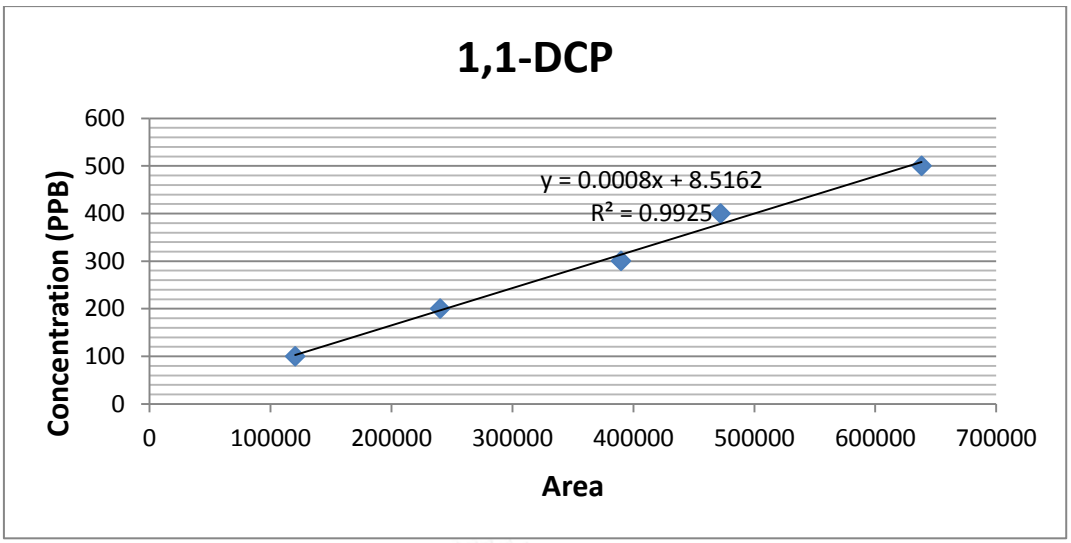


Figure B-3-2 DCAN standard curve for DDCAN

Reduction Test				
	Area	Concentration	mean	SD conc
B1,1 DCP 100	113436.4	99.27		
B1,1 DCP 100	115885.5	101.22	100.24	1.39
B1,1 DCP 300	351270.8	289.53		
B1,1 DCP 300	370514.9	304.93	297.23	10.87
B1,1 DCP 500	618115.6	503.01		
B1,1 DCP 500	607079.5	494.18	498.59	6.24

	Area	Concentration	Mean Conc	SD conc
A1,1 DCP 100	47900.7	46.84		
A1,1 DCP 100	47199.9	46.28		
A1,1 DCP 100	45088.9	44.59	45.90	1.17
A1,1 DCP 300	211686.5	177.87		
A1,1 DCP 300	195434.5	164.86		
A1,1 DCP 300	214066.1	179.77	174.17	8.112059
A1,1 DCP 500	356252.8	293.53		
A1,1 DCP 500	339661.3	280.25		
A1,1 DCP 500	367365	302.41	292.06	11.15
D1,1 DCP 100	98433	87.26		
D1,1 DCP 100	102433	90.46		
D1,1 DCP 100	88661.5	79.45	85.72	5.52
D1,1 DCP 300	332927.6	274.86		
D1,1 DCP 300	341912.3	282.05		
D1,1 DCP 300	344574.7	284.18	280.36	4.88
D1,1 DCP 500	585728.2	477.10		
D1,1 DCP 500	565538.5	460.94		
D1,1 DCP 500	571686.3	465.86	467.97	8.27

B1,1 DCP = Control of 1,1 DCP

A1,1 DCP = Active biofilm with 1,1 DCP

D1,1 DCP = Inctive biofilm with 1,1 DCP

APPENDIX C

Data for the investigation of DBPs Formation Potential

Table C-1 CF Data information for Formation potential investigation

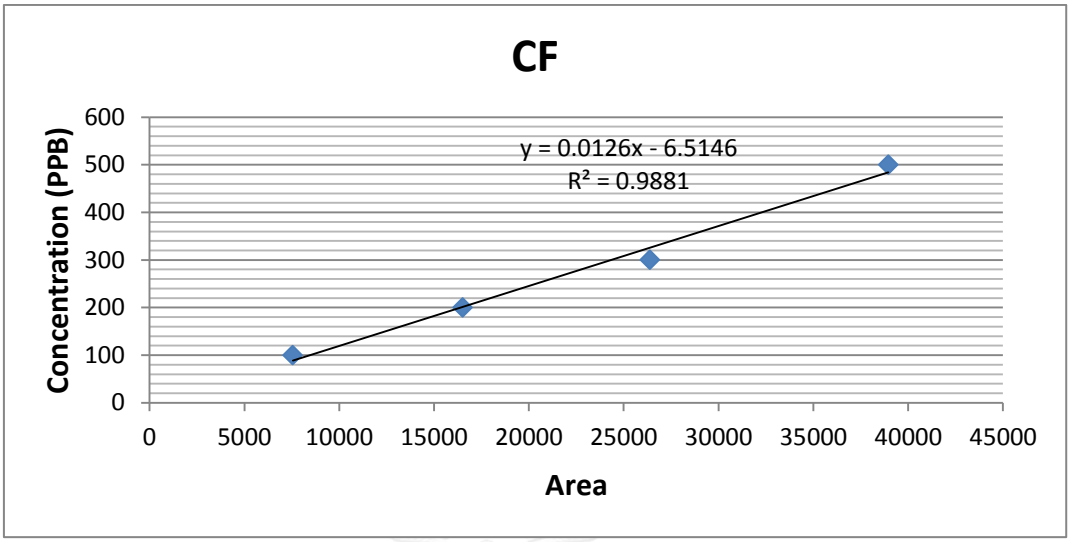


Figure C-1-1 CF standard curve

Std. CF	Area	mean
100	7743.4	
100	7365	7554.2
200	15894.3	
200	17133.9	16514.1
300	26885.2	
300	25871.2	26378.2
400	-	-
400	-	-
500	38065.4	
500	39847.4	38956.4

CF	Area	Average	Concentration	SD	mean Conc overall	SD
EPS1	13570		164.47			
EPS1	11264.3	12417.15	135.42	20.542		
EPS2	10355.1		123.96			
EPS2	15214.4	12784.75	185.19	43.29		
EPS3	10654.2		127.73			
EPS3	10558.4	10606.3	126.52	0.85	143.88	25.14
Biofilm1	11211.2		134.75			
Biofilm1	13424.9	12318.05	162.64	19.72		
Biofilm2	10115.8		120.94			
Biofilm2	12351.4	11233.6	149.11	19.91		
Biofilm3	12197		147.17			
Biofilm3	13004.1	12600.55	157.33	7.19	145.32	15.28

Table C-2 MCAN Data information for Formation potential investigation

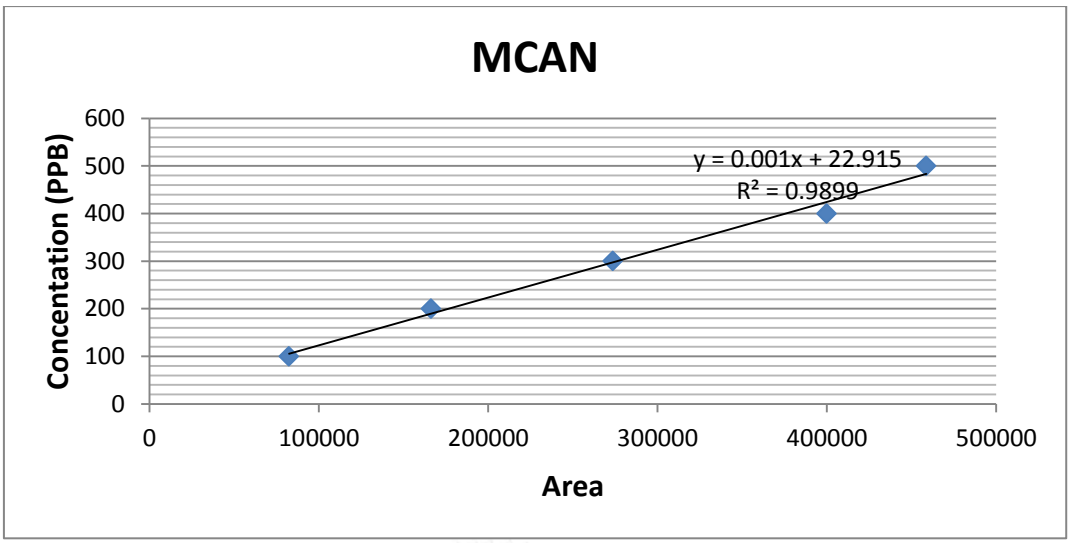


Figure C-2-1 MCAN standard curve

Std. MCAN	Area	mean
100	81870	
100	82707	82288.5
200	169003.2	
200	163496.8	166250
300	257206.3	
300	289803.4	273504.85
400	397949	
400	401852.9	399900.95
500	459895.2	
500	457525	458710.1

MCAN	Area	Average	Concentration	SD	mean Conc overall	SD overall
EPS1	20326.9		43.24			
EPS1	18655.7	19491.3	41.57	1.18		
EPS2	18556.2		41.47			
EPS2	19342.9	18949.55	42.26	0.56		
EPS3	21847.6		44.76			
EPS3	18542.7	20195.15	41.46	2.34	42.46	1.32
Biofilm1	1657.4		24.57			
Biofilm1	1337.3	1497.35	24.25	0.23		
Biofilm2	2867.3		25.78			
Biofilm2	2956.3	2911.8	25.87	0.06		
Biofilm3	1869.6		24.78			
Biofilm3	1189.9	1529.75	24.10	0.48	24.89	0.76

Table C-3 DCAN Data information for Formation potential investigation

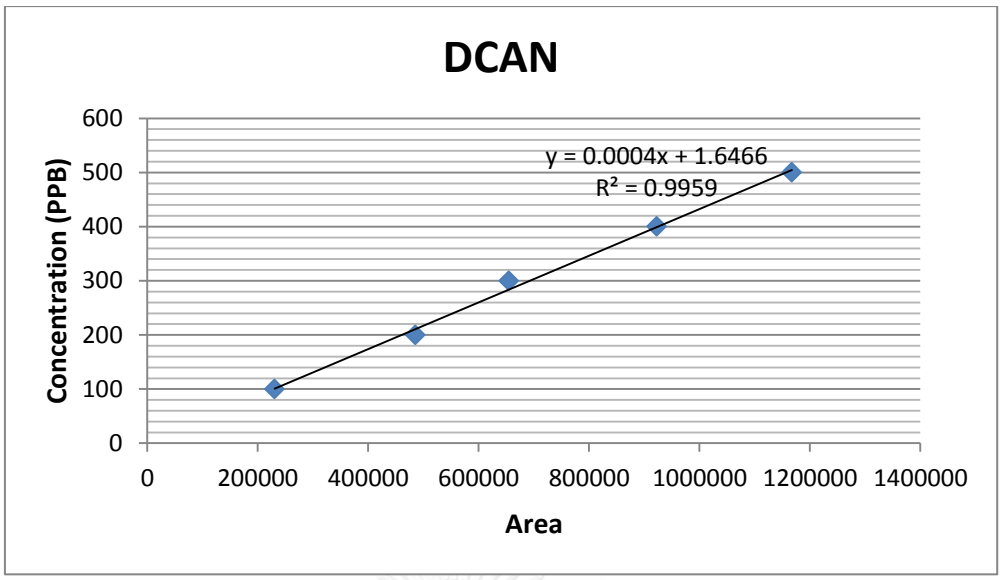


Figure C-3-1 DCAN standard curve

Std. DCAN	Area	mean
100	221393.1	
100	239626.7	230509.9
200	490130.6	
200	481110.5	485620.55
300	648660.1	
300	660490.9	654575.5
400	955682.9	
400	922263.3	922263.3
500	1209044.6	
500	1125414.6	1167229.6

DCAN	Area	Average	Concentration	SD	mean Conc overall	SD overall
EPS1	18812.4		9.17			
EPS1	18357.2	18584.8	8.99	0.13		
EPS2	18480.7		9.04			
EPS2	19221.3	18851	9.34	0.21		
EPS3	22297		10.57			
EPS3	23442.8	22869.9	11.024	0.32	9.69	0.878009
Biofilm1	9297		5.36			
Biofilm1	8442.8	8869.9	5.023	0.24		
Biofilm2	19159		9.31			
Biofilm2	17741	18450	8.74	0.40		
Biofilm3	21862.6		10.39			
Biofilm3	21926.9	21894.75	10.41	0.02	8.21	2.42366

Table C-4 TCAN Data information for Formation potential investigation

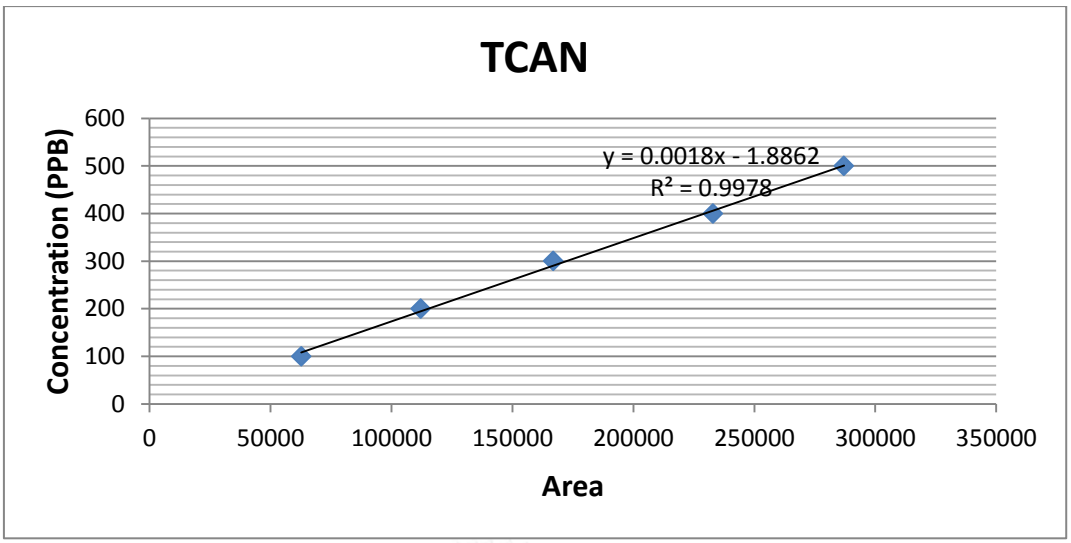


Figure C-4-1 TCAN standard curve

Std. TCAN	Area	mean
100	61702.6	
100	63691.9	62697.25
200	112659.9	
200	111486.5	112073.2
300	168415.9	
300	165441.5	166928.7
400	224089.7	
400	232858.6	232858.6
500	-	-
500	287097.5	287097.5

TCAN	Area	Average	Concentration	SD	mean Conc overall	SD overall
EPS1	2965.8		3.45			
EPS1	2676.2	2821	2.93	0.37		
EPS2	3164.2		3.81			
EPS2	3238.4	3201.3	3.94	0.09		
EPS3	2898.6		3.33			
EPS3	2975.2	2936.9	3.47	0.10	3.49	0.36
Biofilm1	72		0			
Biofilm1	59.1	65.55	0			
Biofilm2	ND		-			
Biofilm2	57.3	57.3	0			
Biofilm3	73.8		0			
Biofilm3	78.6	76.2	0	0	0	0

Table C-5 1,1 DCP Data information for Formation potential investigation

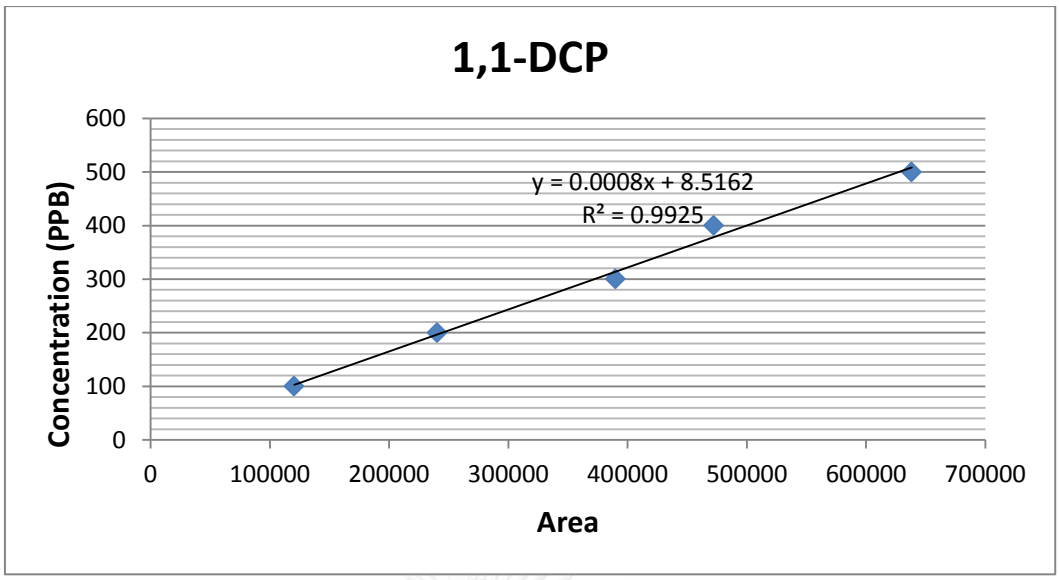


Figure C-5-1 1,1 DCP standard curve

Std.1,1 DCP	Area	mean
100	110398.7	
100	130506.6	120452.65
200	249677.5	
200	231126.6	240402.05
300	393843.8	
300	385712.8	389778.3
400	472413.5	
400	472082.5	472248
500	638271.9	
500	-	638271.9

1,1 DCP	Area	Average	Concentration	SD	mean Conc overall	SD overall
EPS1	21892.6		26.03			
EPS1	23832.6	22862.6	27.58	1.10		
EPS2	22422.3		26.45			
EPS2	23930.3	23176.3	27.66	0.85		
EPS3	23115.2		27.01			
EPS3	22106.1	22610.65	26.20	0.57	26.82	0.70
Biofilm1	1342.1		9.59			
Biofilm1	1248.1	1295.1	9.51	0.05		
Biofilm2	1712.3		9.89			
Biofilm2	2827.2	2269.75	10.78	0.63		
Biofilm3	1155.6		9.44			
Biofilm3	1189.9	1172.75	9.47	0.02	9.77	0.51

Table C-6 1,1,1 TCA Data information for Formation potential investigation

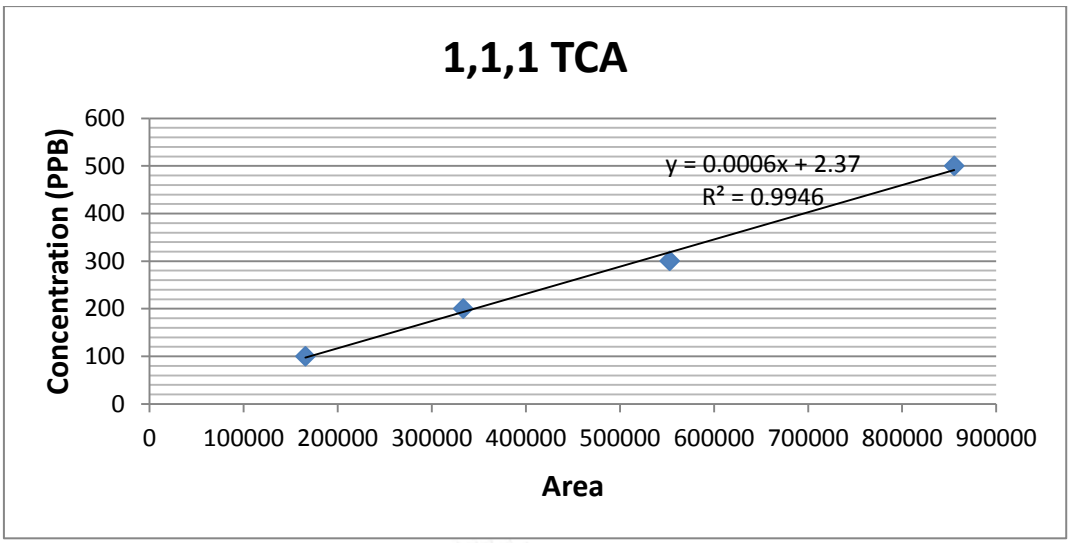


Figure C-6-1 1,1,1 TCA standard curve

Std. 1,1,1 TCA	Area	mean
100	160558.3	
100	170946	165752.15
200	348701.7	
200	318620.4	333661.05
300	548711.1	
300	557112.9	552912
400	mistake	
400	mistake	
500	831590.4	
500	879057.6	855324

1,1,1 TCA	Area	Average	Concentration	SD	mean Conc overall	SD overall
EPS1	2760		4.02			
EPS1	3391.6	3075.8	4.40	0.27		
EPS2	2254.5		3.72			
EPS2	2546.5	2400.5	3.89	0.12		
EPS3	3317.2		4.36			
EPS3	3039	3178.1	4.19	0.12	4.10	0.27
Biofilm1	199.6		2.49			
Biofilm1	56	127.8	2.40	0.06		
Biofilm2	147.6		2.46			
Biofilm2	308.5	228.05	2.56	0.07		
Biofilm3	49.8		2.40			
Biofilm3	75	62.4	2.42	0.01	2.45	0.06

Table C-7 MCAA Data information for Formation potential investigation

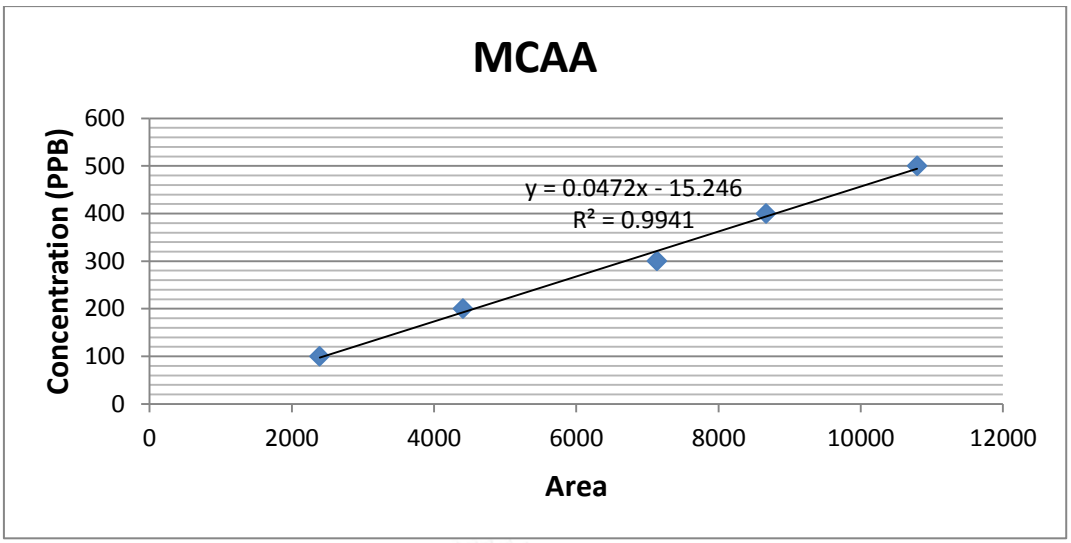


Figure C-7-1 MCAA standard curve

Std. MCAA	Area*DF*CF	mean	% Recovery
100	2381.34		100.67
100	2404.41	2392.88	105.41
200	4261.18		105.41
200	4551.45	4406.31	114.76
300	7612.85		93.98
300	6656.62	7134.74	105.03
400	9241.49		94.74
400	8098.79	8670.14	109.83
500	11053.42		105.16
500	10530.49	10791.95	100.94

MCAA	Area*DF*CF	Average	Concentration	SD	mean Conc overall	SD overall
EPS1	1121.57		37.69			
EPS1	1270.87	1196.22	44.74	4.98		
EPS2	1259.07		44.18			
EPS2	990.46	1124.77	31.50	8.96		
EPS3	1307.50		46.46			
EPS3	1144.14	1225.82	38.75	5.45	40.55	5.63
Biofilm1	845.51		24.66			
Biofilm1	904.75	875.13	27.45	1.97		
Biofilm2	1013.20		32.57			
Biofilm2	1120.39	1066.79	37.63	3.57		
Biofilm3	813.38		23.14			
Biofilm3	1099.23	956.30	36.63	9.54	30.35	6.16

Table C-8 DCAA Data information for Formation potential investigation

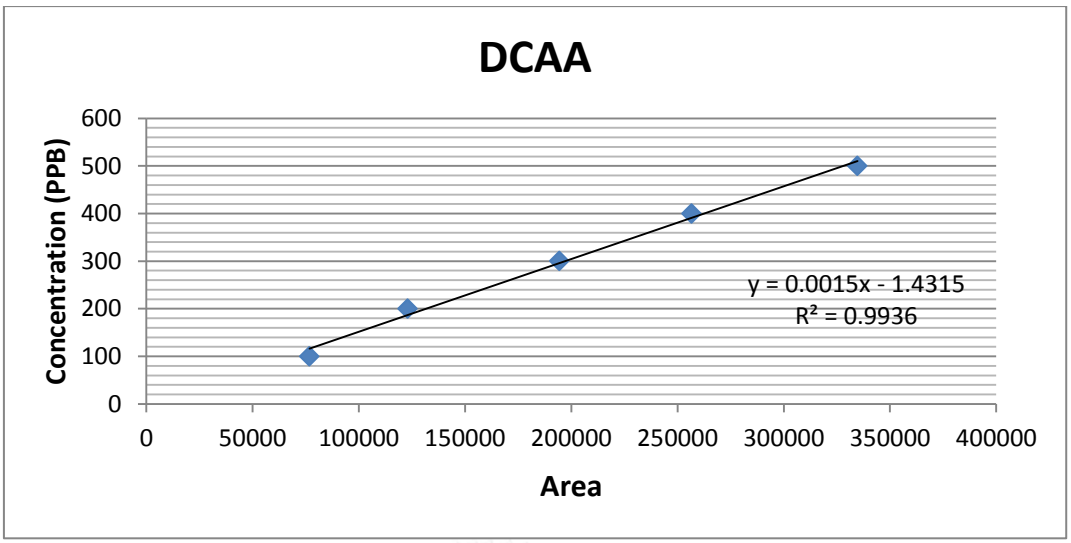


Figure C-8-1 DCAA standard curve

Std. DCAA	Area*DF*CF	mean	% Recovery
100	75403.26		95.70938
100	78073.74	76738.5	91.15216
200	120006.4		99.96853
200	125824.6	122915.5	105.1224
300	194510.6		103.4965
300	194268.1	194389.4	105.5507
400	259577.9		99.93621
400	253667	256622.4	110.3471
500	321996.9		111.8213
500	347362.8	334679.9	93.34139

DCAA	Area*DF*CF	Average	Concentration	SD	mean Conc overall	SD overall
EPS1	59549.81		87.89			
EPS1	59483.95	59516.88	87.79	0.069		
EPS2	62150.07		91.79			
EPS2	74582.77	68366.42	110.44	13.18		
EPS3	73354.57		108.60			
EPS3	59855.73	66605.15	88.35	14.31	95.81	10.73
Biofilm1	33628.77		49.01			
Biofilm1	29913.23	31771	43.43	3.94		
Biofilm2	60773.47		89.72			
Biofilm2	54809.7	57791.58	80.78	6.32		
Biofilm3	41011.98		60.08			
Biofilm3	39141.35	40076.66	57.28	1.98	63.38	18.16

Table C-9 TCAA Data information for Formation potential investigation

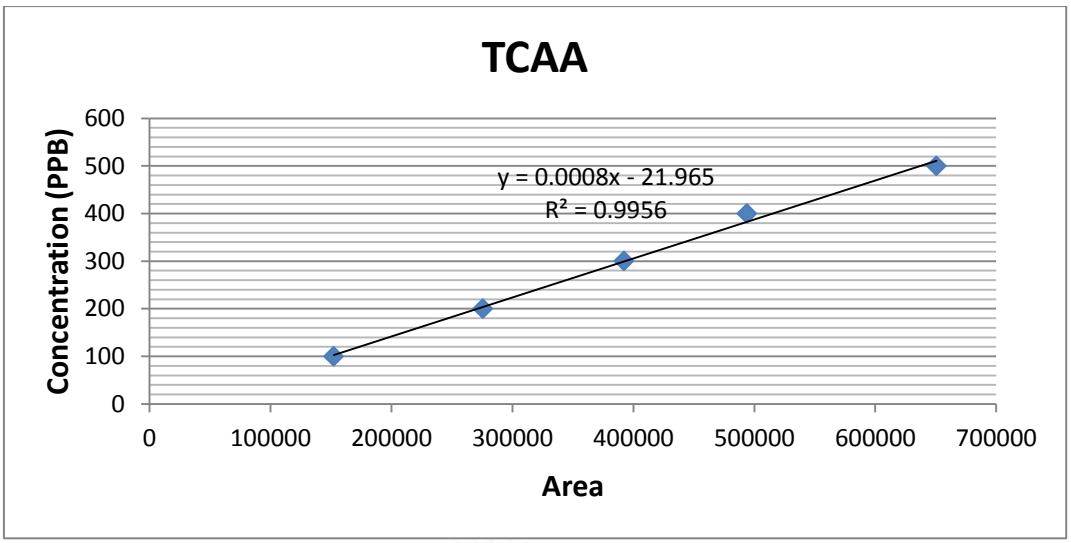


Figure C-9-1 TCAA standard curve

Std. DCAA	Area*DF*CF	mean	% Recovery
100	-		-
100	152357.3	152357.3	106.40
200	281568.1		99.96
200	269433.7	275500.9	104.24
300	387394.9		94.70
300	397123.8	392259.4	95.57
400	-		-
400	493974.2	493974.2	110.34
500	642430.1		103.80
500	658865.2	650647.6	88.58

DCAA	Area*DF*CF	Average	Concentration	SD	mean Conc overall	SD overall
EPS1	117858.7		72.32			
EPS1	126430.6	122144.7	79.17	4.85		
EPS2	222584.9		156.10			
EPS2	182068.3	202326.6	123.68	22.92		
EPS3	159925		105.97			
EPS3	149422.3	154673.7	97.57	5.94	95.81	30.81
Biofilm1	116748.4		71.43			
Biofilm1	94143.27	105445.8	53.34	12.78		
Biofilm2	106113.5		62.95			
Biofilm2	136334.9	121224.2	87.10	17.09		
Biofilm3	107188.5		63.78			
Biofilm3	105325.9	106257.2	62.29	1.05	63.38	11.48

VITA

Mr. Kittithach was born on November 2, 1990 in Bangkok province. He graduated Bachelor's degree of Environmental Engineering from Chulalongkorn University. After that he continued study in Master's degree of science in Environmental management, Chulalongkorn University.

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