CHAPTER IV

RESULTS AND DISCUSSIONS

4.1 Effects of sludge, nutrients and carbon source on PCE reductive dechlorination

A preliminary study was carried out to determine the effects of sludge seed, mineral nutrients, and carbon source supplementations on PCE reductive dechlorination in soil slurry reactors. Figure 4.1 showed the difference of PCE removal efficiency under various conditions. Total amount of PCE (100 mg-PCE/kg-soil) remained in the reactor of which nothing was added while PCE removal efficiencies of nutrient addition and sludge seed addition reactors were 17.19% and 21.4 %, respectively (Figure 4.1).

This suggested that the contaminated soil can not remove PCE without the addition of nutrient or sludge. When adding both nutrient and sludge, the efficiency of PCE removals was raised up to 42.24%. Although, the sludge and nutrient were found to be important for anaerobic reductive dechlorination, they still had low PCE removal efficiency.

Best PCE removal efficiency (88.7%) was finally achieved in the case of adding the three additives: nutrient, sludge seed and carbon source altogether into the

reactor. Carbon source as well as mineral nutrients and sludge seed are proved to be necessary for the effective PCE dechlorination.

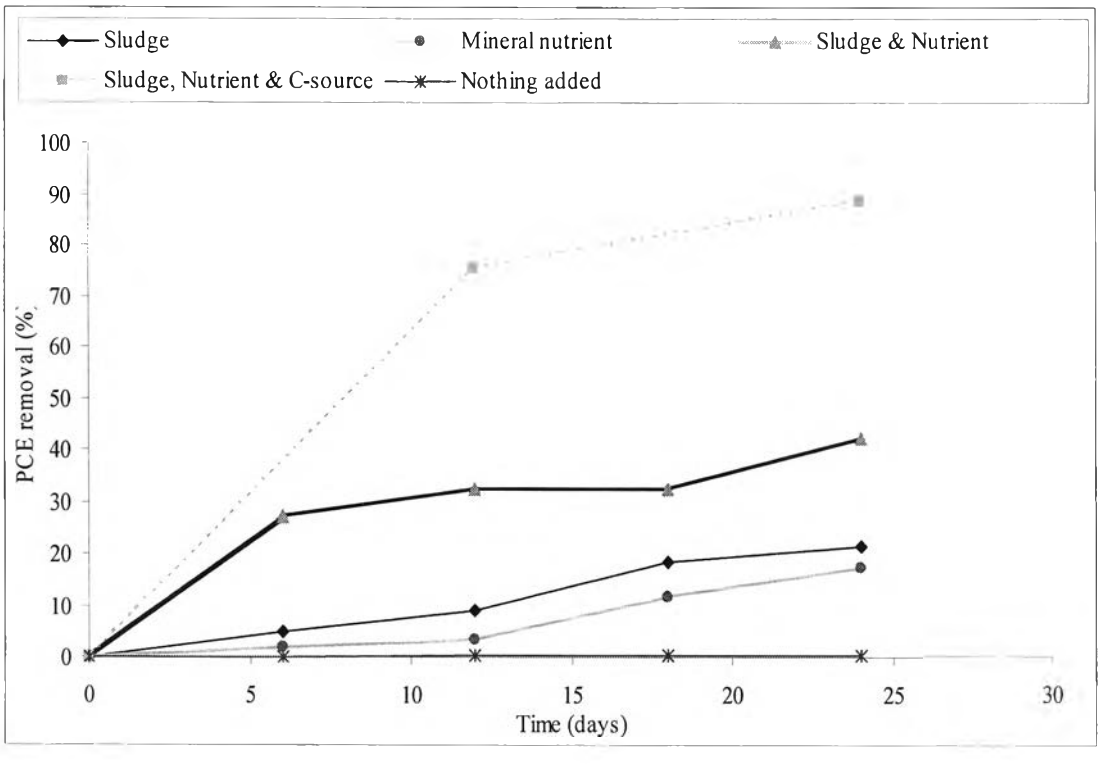

Figure 4.1 Effects of various supplements on PCE dechlorination

This experiment showed that unacclimated sludge from food industry enables to use as seed in soil slurry rector and can enhance the effectiveness on PCE removal under anaerobic condition. It is consistent with Tungmee (2002), she reported that unacclimated sludge enable to remove HCB using glucose as electron donor.

4.2 Optimum concentrations of food grade substrate to enhance PCE dechlorination

4.2.1 Decrease of PCE Dechlorination

In this experiment, unacclimated sludge from a UASB treatment plant of Leo Food industry was applied to PCE contaminated (100 mg-PCE / kg-soil) soil slurry reactors and incubated for 24 days to conduct the dechlorination. The composition of sand: silt: clay in soil sample was 85.3: 11.6: 3.1. The unacclimated sludge was added as seed for anaerobic bacteria in the system. Various concentrations of glucose and soybean oil were used in the experiment as electron donor. Glucose and soybean oil are food grade substrates, in which their electron providing activities and PCE removal efficiency will be compared with related waste substrates in Figure 4.3. After 24 days, PCE was found to be decreased from the soil slurry with PCE dechlorination efficiency of 54.24 % to 99.47 % as described in Table 4.1.

From Table 4.1, PCE removal efficiencies in reactors with 500-10,000 mg-glucose /kg-sludge were ranged from 99.16% - 99.47% while those with 500-10,000 mg-soybean oil /kg-sludge had 98.28% - 99.05% PCE removal.

Table 4.1 PCE dechlorination efficiency when using glucose and soybean oil as electron donors within 24 days.

Organic Substrate	Concentration of organic substrate (mg/kg-sludge)	% PCE removal within 24 days (Triplicate)
1. No organic substrate	-	54.24 ± 1.30
2. Glucose	500	99.16 ± 0.76
	1,000	99.41 ± 0.28
	5,000	99.46 ± 0.27
	10,000	99.47 ± 0.14
3. Soybean oil	500	98.96 ± 0.55
	1,000	98.83 ± 0.23
	5,000	99.05 ± 0.65
	10,000	98.28 ± 0.16

Comparing to the other studies the obtained PCE removal efficiency of this study was superior are probably due to higher amount of carbon supplementation. Doong and Wu (1994) showed the percent PCE removal in fed-batch reactors when supplemented with 10 mg/l glucose was 66.9%. The PCE removal efficiency was increased to 75.2% when glucose addition concentration was increased to 30 mg/l. These suggested that organic substrate concentration can effect on the transformation of the chlorinated hydrocarbons. Higher concentration of the supplied organic substrate concentrations can induce larger PCE removal in system. However, the highest PCE removal will be achieved when an adequate organic carbon were supplied to the system. Lee et al (2003) reported that PCE was decreased by 96% when the soybean oil was supplied in the contaminated soil. Not only the appropriate

carbon source addition but other appropriate conditions such as soil structure, pH, nutrient and temperature were also necessary to enhance the PCE removal.

From the results, glucose appeared to be a slightly better PCE reductive dechlorination comparing to soybean oil. It is interested that both food grade substrate act as electron donor for PCE reductive dechlorination. The reason for this may be explained in terms of molecular structures of these two substrates. Glucose's formula is $C_6H_{12}O_6$ while the general molecular of edible oil is $CH_3\ (CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7CO_2H$. Because glucose composed with less hydrogen atom comparing with soybean oil but glucose is easier to degrade than that soybean oil therefore at the highly concentration, more degradable glucose can provide highly hydrogen atom for reductive dechlorination. While soybean oil is harder to degrade by the anaerobic bacteria in a short period (Lee et al., 2003) but soybean oil composed with a large amount of hydrogen atoms a molecule of soybean oil would release more hydrogen atoms than that of glucose if only less soybean oil degradation occurred. These hydrogen atoms from soybean oil will then replace chlorine atoms in PCE molecule. However, the result might be different for long period operation or for the comparison between substrates of more complex molecule such as molasses, flour and oil waste. Consequently, the dechlorination process of PCE under soybean oil addition could be largely enhanced if there were enough reaction time. This would bring higher dechlorination efficiency than that in the case of glucose addition. The incubation time period of this study was 24-days because many researchers recorded that PCE can dechlorinate within 25 days period (Ferguson and Pietari, 1999; Leahy and

Shreve, 1999; Kao et al., 2003). In the other hand this incubation time was designed in the short time period because some limitation of laboratory time period.

No significant difference among dechlorination efficiencies was found within the same-type substrate, 500 – 10,000 mg-glucose/kg-sludge (represent COD concentration of substrate around 250 - 5,000 mg/L) and 500 – 10,000 mg-soybean oil/kg-sludge (represent COD concentration ranged 350 – 6,500 mg/L). These COD concentration is consistent with Tungmee (2002) in which she used glucose and the combination of acetic acid, butyric acid and propionic acid (as lipid) as the organic substrate 3,000 mg/l of glucose and 2,320 mg/l of lipid are equal to COD concentration of 3,000 mg/l. These concentrations are therefore appropriate to support respiration process and were considered to be enough for PCE dechlorination.

It should be noticed that PCE dechlorination efficiency of 54.24 % was recognized in the control condition (no carbon source addition). The reason is probably because anaerobic bacteria could used soil organic carbon and the supplied mineral nutrients for growth and then produce electron and hydrogen for PCE dechlorination. Meanwhile, the amount of soil organic carbon was minimal thus we need to supply the soil with external organic substrate to the enhance dechlorination process.

PCE concentrations in the soil slurry coupled with reaction times were shown in figure 4.2 and figure 4.3. At the earlier period (0 to 6^{th} days), PCE from every set were rapidly dechlorinated. From figure 4.1, 99.16 to 99.47 % of PCE were

dechlorinated under glucose addition within 6 days of reaction time. Similar to the case of soybean oil addition, 98.28 to 99.05% of PCE in the reactor was degraded after the 6th day (see figure 4.3).

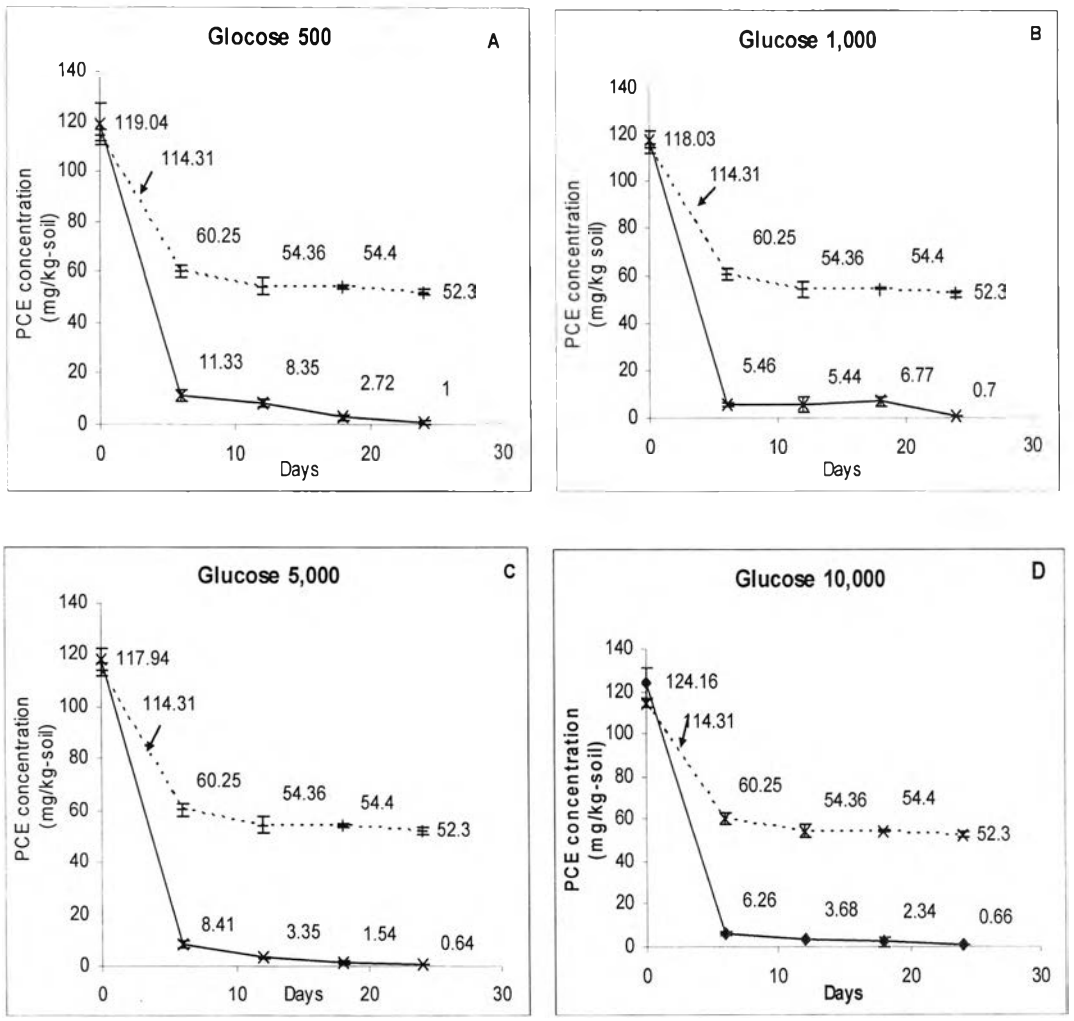

Figure 4.2 Comparison of PCE removal efficiency between no carbon source addition (dot line) and various concentration of glucose (solid line); A = 500 mg/kg-soil, B = 1,000 mg/kg-soil, C = 5,000 mg/kg-soil, and D = 10,000 mg/kg-soil

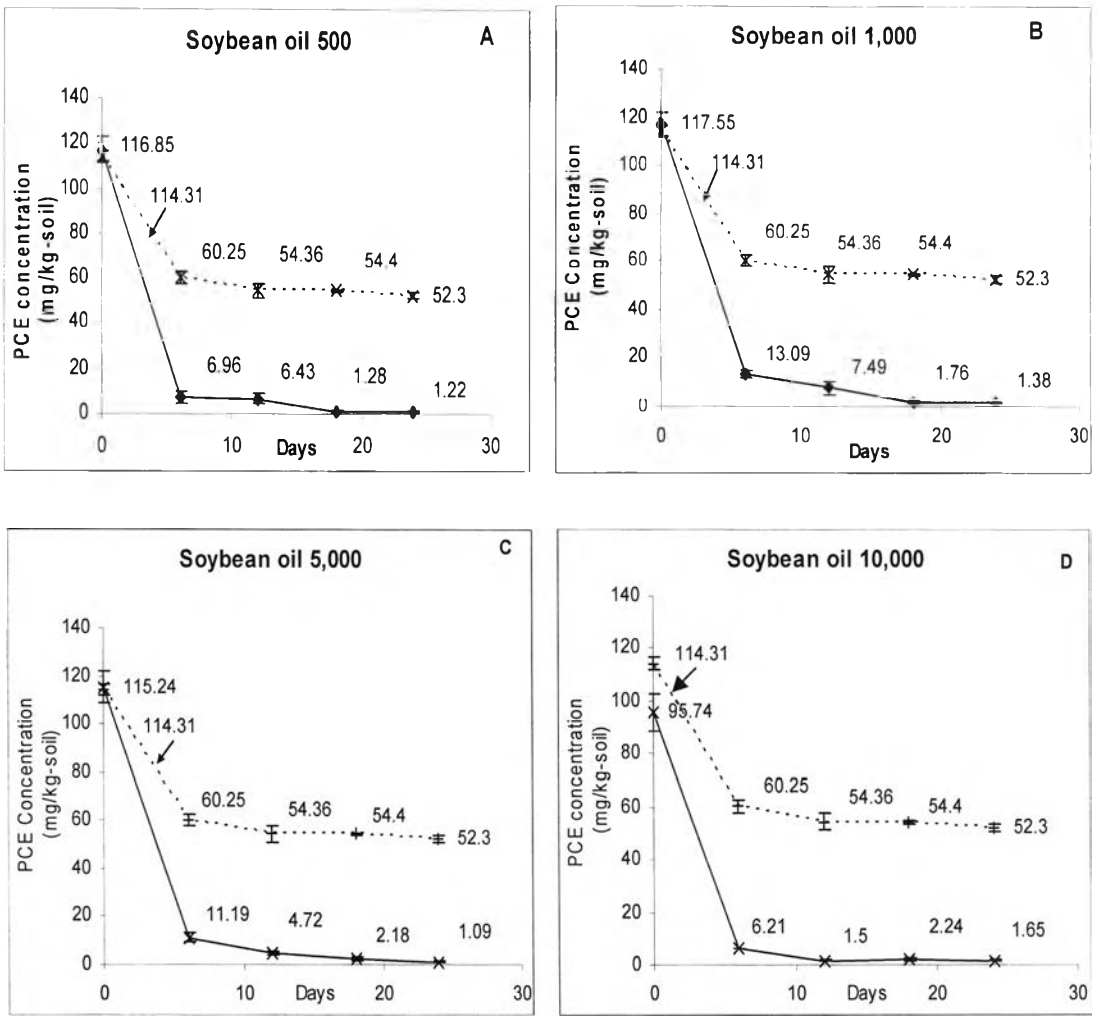

Figure 4.3 Comparison of PCE removal efficiency between no carbon source addition (dot line) and various concentration of soybean oil (solid line); A = 500 mg/kg-soil, B = 1,000 mg/kg-soil, C = 5,000 mg/kg-soil, and D = 10,000 mg/kg-soil

Consequently, the reaction time needed for PCE dechlorination should not be less than 6 days. Comparing to the study of Kao et al., 2003, they reported that PCE can be rapidly removed from the system within 5 to 10 day and PCE removal was appropriately 96%. After 10 days, the PCE was slowly removed and the finally PCE

removal in the system was 98.8%. It is noticed that in the appropriate condition, PCE can be rapidly removed in the earlier time period. For the control set (no carbon source addition) rapid dechlorination of PCE were also observed at the same period but the PCE removal rates were less than other sets. This implies the lack of hydrogen atom when no carbon sources were added to the process.

According to the above result, it is still difficult to select two best concentrations of both food grade substrates for applying to the next waste addition experiments. However, more information used for the selection could be received from the analysis of PCE intermediates in was carried out in the next step.

4.2.2 Production of PCE Intermediates, TCE and DCE

There were reports that PCE always persist under aerobic condition, while it can be dechlorinated into harmless product under anaerobic condition (Lee, 2002; Ferguson and Pietari, 1999). Trichloroethene, dichloroethene, vinyl chloride and end product are the intermediates of PCE which were dechlorinated under anaerobic condition. The intermediates were detected by gas chromatography, with difference retention times on GC chromatogram.

This part of the study designed to observe the PCE intermediates produced in the system in order to confirm the anaerobic reductive dechlorination and choose two best concentrations of food grade substrates (glucose and soybean oil) for applying to the next experiments.

1. Glucose act as substrate

From figure 4.4, TCE can be detected from all treatments. There were found that 10,000 mg-glucose/kg-sludge gave the highest TCE production at day 18 and the amount of TCE was decreased afterward.

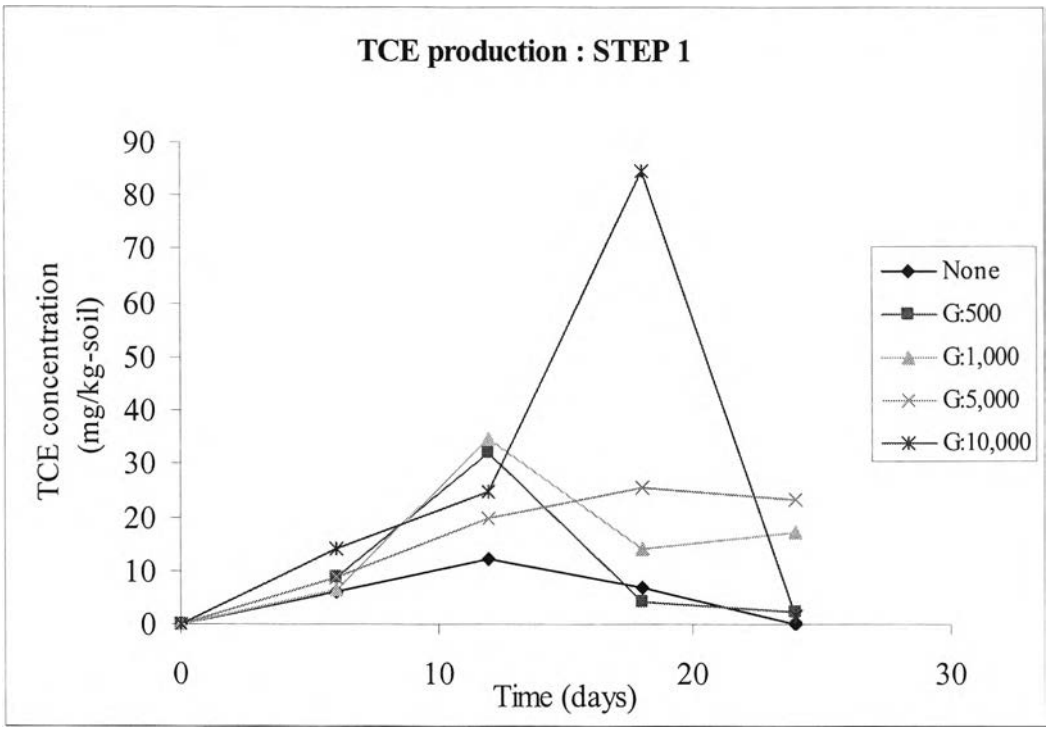

Figure 4.4 Total TCE production (mg/kg-soil) using glucose as substrate.

DCE can only be detected from reactors containing 5,000 and 10,000 mg-glucose/kg sludge (Figure 4.5). Other intermediates i.e. vinyl chloride, ethene, ethane were not found in this experiment. This confirmed that the PCE in the two sets of reactors were dechlorinated to less chlorinated compounds. Thus, 5,000 and 10,000

mg-glucose/ kg-sludge were selected to apply for molasses concentration in the next experiment.

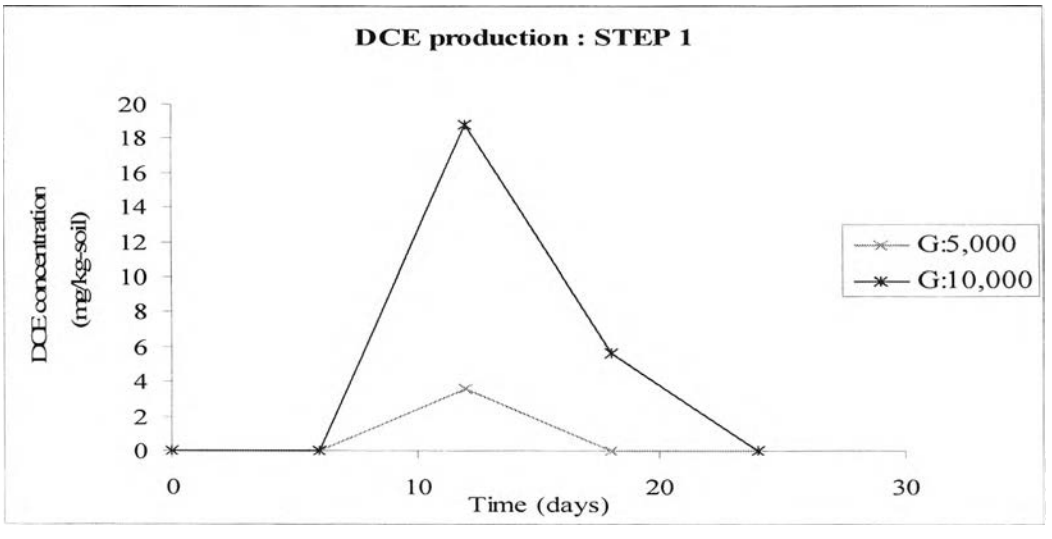

Figure 4.5 Total DCE production (mg/kg-soil) using glucose as substrate

2. Soybean oil acts as substrate.

From figure 4.6, TCE can be detected from all various soybean oil concentrations sets as same as it was observed in the case of glucose addition. However, there were only two best concentrations of which were 500 mg-soybean oil/kg-sludge and 5,000 mg-soybean oil /kg-sludge that could provide higher TCE production and DCE production (Figure 4.7). Thus, 500 and 5,000 mg-soybean oil/ kg-sludge were selected as the concentration to apply for the oil waste addition experiments. Other intermediates i.e. vinyl chloride, ethene, ethane were not detected from this experiment.

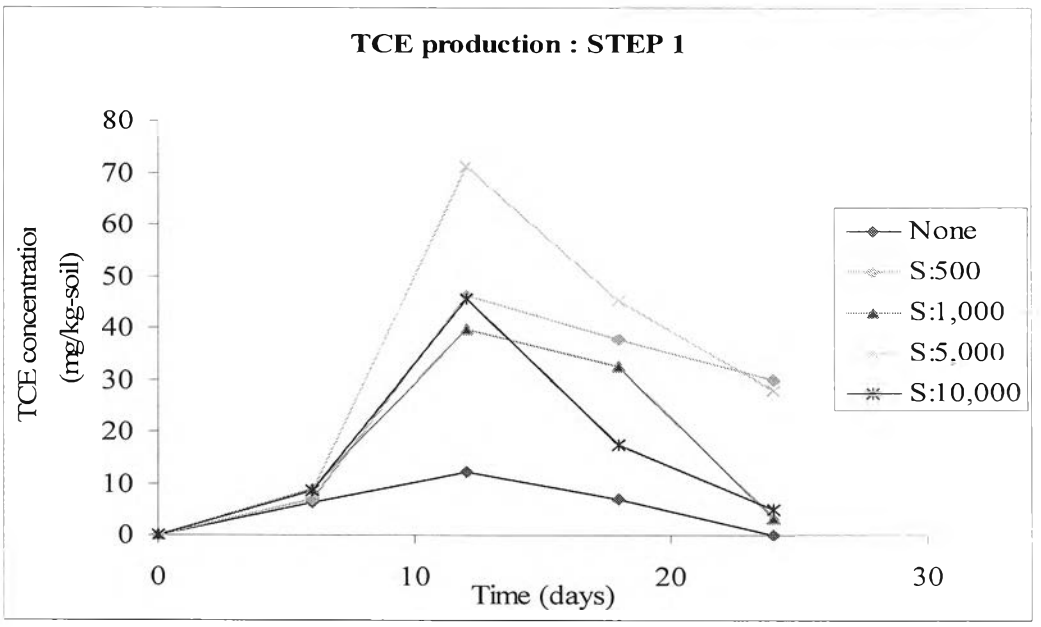

Figure 4.6 Total TCE production (mg/kg-soil) using soybean oil as substrate.

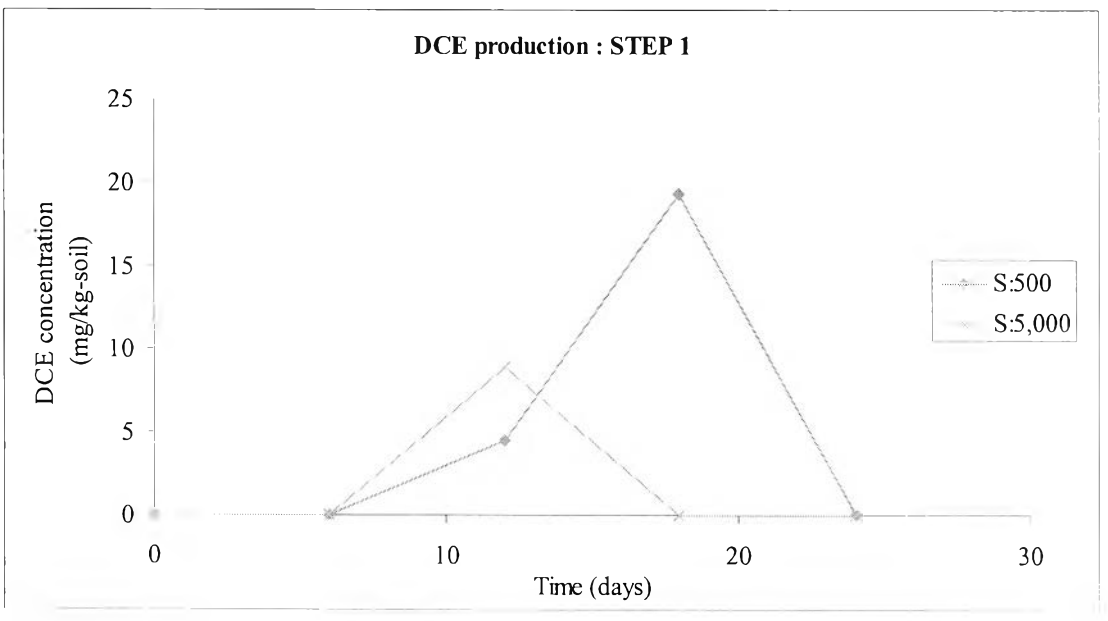

Figure 4.7 Total DCE production (mg/kg-soil) using soybean oil as substrate.

4.2.3 Total Gas Production

The amount of gas produced in soil slurry reactors was measured from the elevation of septum every 6 day. The septum which connected to the soil slurry vial was pushed up by gas produced from microbial activity during reductive dechlorination. There were assumed that less gas production leaked from soil slurry reactors because of silicone glue used to seal the leakage between glass vial and plastic syringe. However, the volume of gas elevation does not always represent the actual gas production or indicate the level of dechlorination progress because of some limitation. For example, the friction between septum and inner surface of syringe resulted in underestimation of total gas production. Although there is no friction, larger volume of gas production dose not always directly indicate better progress of dechlorination due to the fact that the gases such as CO_2, CH_4 and H_2S are produced in respiratory process of bacteria which continue undergoing no matter there is dechlorination reaction or not. Therefore, the result of gas production will be used only to confirm the respiratory process of anaerobic bacteria.

The dechlorination is therefore considered to take place in all sets of experiment. The control set (no carbon source addition) seemed to produce the least volume of gas as shown in figure 4.8.

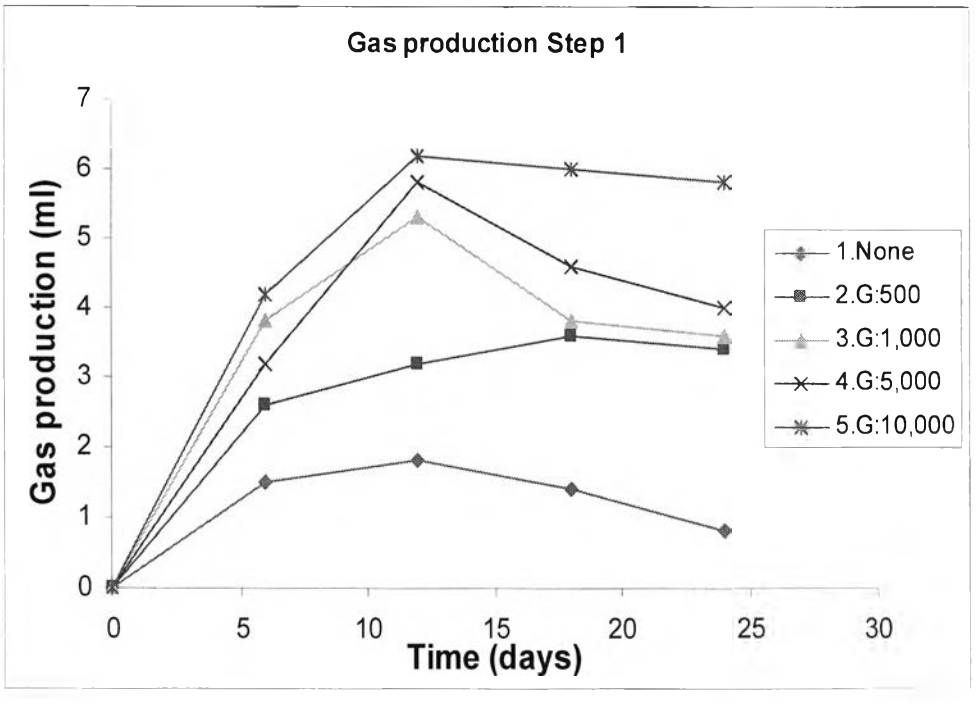

Figure 4.8 Total gas production (ml) using glucose as substrate.

Figure 4.8 showed the total gas production during the experiment using glucose as carbon source. The higher concentrations of glucose influenced the higher amount of gas production. These results suggested that organic digestion process was occurred and our soil slurry reactors were indeed an anaerobic system.

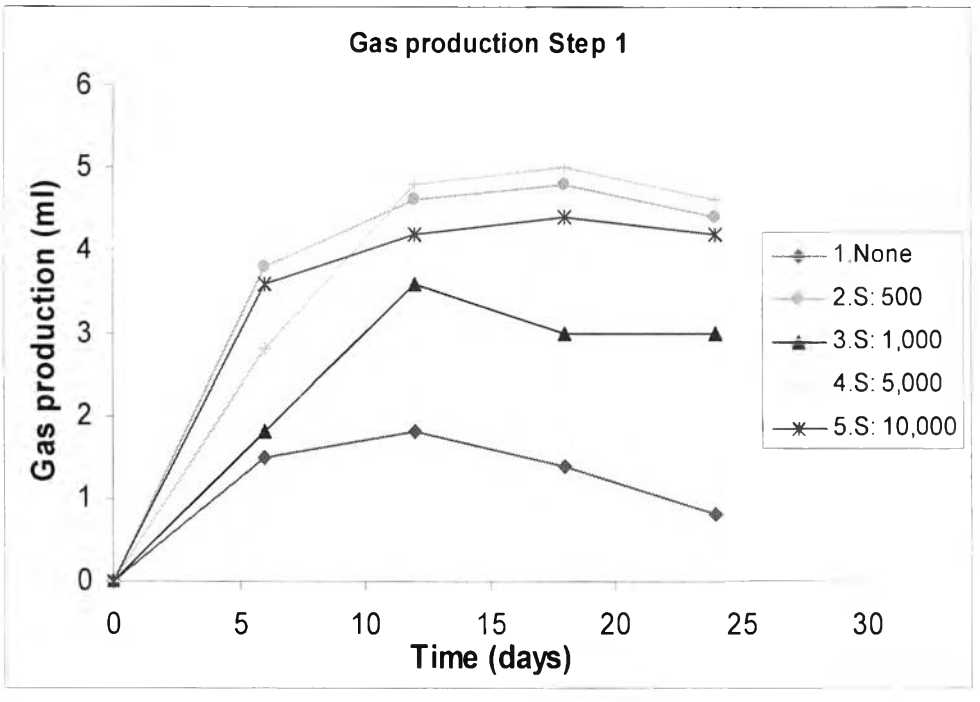

Figure 4.9 Total gas production (ml) using soybean oil as substrate.

From figure 4.9, it was noticed that two sets of soybean oil at concentration 500 and 5,000 presented the largest volume of gas production. The reason is because glucose easier to degrade therefore more carbons in glucose was consumed under the food digestion process, this carbon consumption lead to highly gas production and cell growth.

4.2.4 Chloride Production

Under anaerobic conditions, reductive dehalogenation is the dominant mechanism for chloride removal. Reductive dechlorination of chlorinated organic compounds takes place in reduced environments such as deep soils and sediments and is mediated by native microbial consortia acclimated to the existing contaminants. The result of reductive dechlorination is less chlorinated compounds congener, which is less toxic, less likely to bioaccumulate, more soluble and volatile than the polychlorinated compounds. Thus more mobile and susceptible for microbial attack are produced (Adriaens and Vogel, 1995).

Anaerobic reductive dechlorination occurs in a microbe's respiratory processes during food digestion. H^+ is produced in the acidogenesis process and chloride molecules in the structure of chlorinated compounds are replaced with hydrogen molecule until the end of reaction. Thus more free residual chlorides represent better progress of dechlorinaiton.

The amount of chloride released during dechlorination is measured and show in Figure 4.10. The result was used to ensure the theory of PCE reductive dechlorination under anaerobic condition. From figure 4.10, chloride in the system increased rapidly after 5 days and provided the highest accumulated after 24 days. The increasing chloride is consistent with the decreasing of PCE in section 4.2.1. The high chloride productions were presented in the 10,000 mg-glucose / kg-sludge set, 500 mg-soybean oil / kg-sludge, 5,000 mg-glucose / kg-sludge and 5,000 mg-soybean

oil / kg-sludge, respectively. This is because chloride, in PCE molecule, was replaced by hydrogen atom from the respiratory process. Therefore, the free residual chloride will be accumulated abundantly in the system.

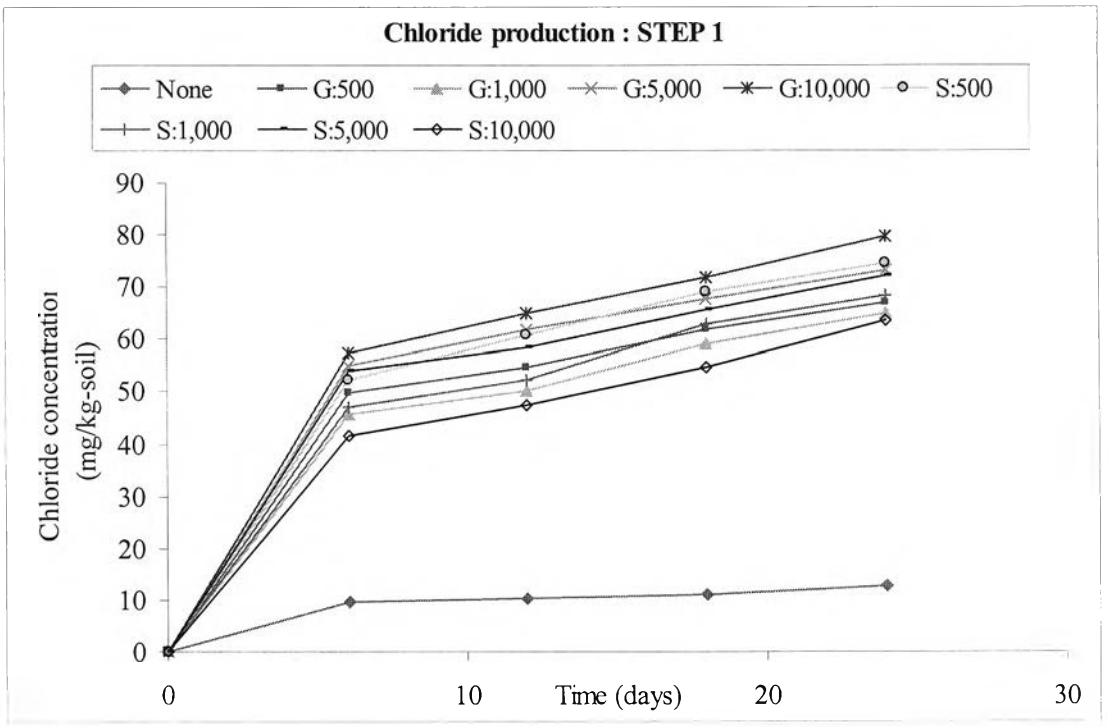

Figure 4.10 Chloride productions from the first experiment

4.3 Optimum concentration of molasses and edible oil wastes to enhance PCE dechlorination

4.3.1 Decrease of PCE Dechlorinations

In this experiment, unacclimated sludge from a UASB treatment plant of Leo Food industry, Chiang Mai province was applied to PCE contaminated soil (100 mg-PCE / kg-soil) and placed in the closed vial for 24 days to conduct the dechlorination. Soil sample composed with 85.3: 11.6: 3.1 of sand: silt: clay ratio. Various concentrations of molasses and wastes oil were used in the experiment as organic substrates. The two best concentrations of glucose (5,000 and 10,000 mg/kg) were applied for molasses set and those of soybean oil (500 and 5,000 mg/kg) were applied for the rest of three oil wastes in the experiment. After 24 days, PCE was found to be decreased from the soil slurry with dechlorination efficiency of 97.47 % to 99.46 % as described in Table 4.2.

The results from molasses were as effective as Kao et al. (2003), reported that cane molasses can be used as the primary substrate for PCE removal. They were found that PCE removal occurred rapidly within 5 to 10 day and the PCE removal was 98.8%.

Meanwhile, the best three substrates for PCE removal efficiency were used lard (500 mg/kg-sludge), used soybean oil (5,000 mg/kg-sludge) and waste oil from Leo Food (5,000 mg/kg-sludge).

Table 4.2 Efficiency of PCE removal using molasses and oil wastes as organic substrate within 24 days.

Organic Substrate	Concentration of organic substrate (mg/kg-sludge)	% PCE removal within 24 days (Triplicate)
1. Molasses	5,000	98.82 ± 0.84
2. Molasses	10,000	97.47 ± 1.34
3. Used lard	500	99.41 ± 0.65
4. Used lard	5,000	98.13 ± 0.51
5. Used soybean oil	500	99.10 ± 0.69
6. Used soybean oil	5,000	99.46 ± 1.65
7. Oil wastes from Leo Food	500	99.00 ± 0.26
8. Oil wastes from Leo Food	5,000	99.46 ± 0.74

Figure 4.11 showed the amount of PCE residual in reactors of the above three experiments during 24 day-incubation period PCE in system were dechlorinated rapidly during the first 6 days period and slowly dechlorinated after that.

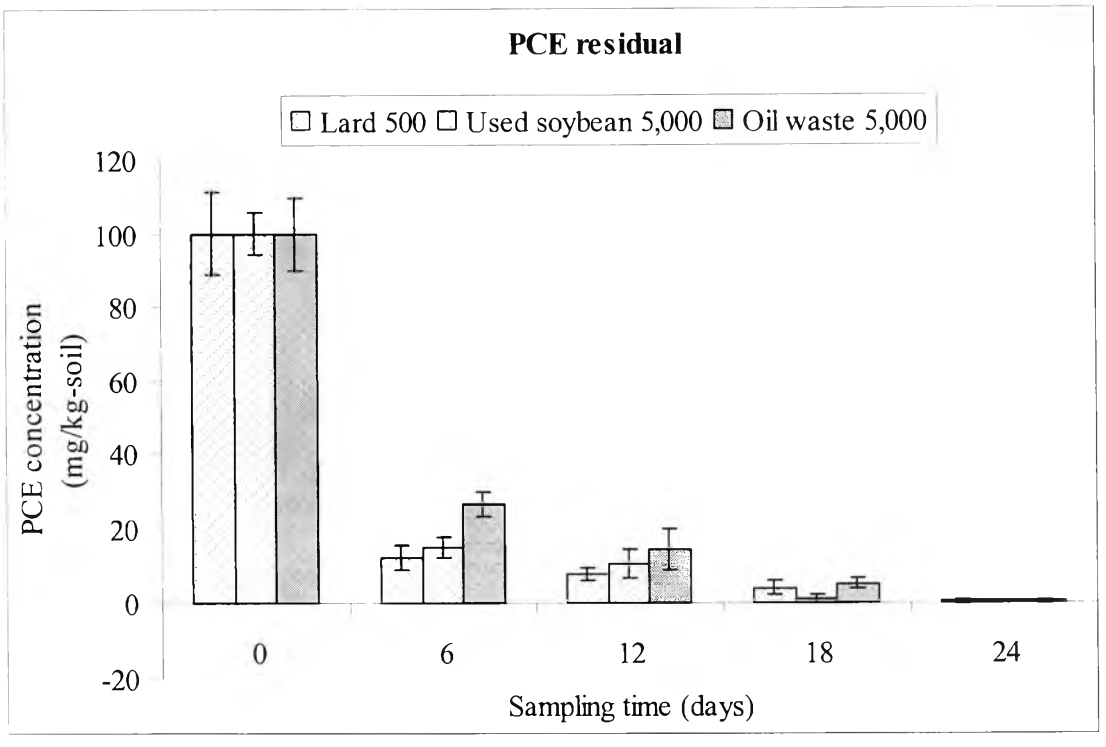

Figure 4.11 PCE residual from three wastes act as carbon source.

Total amount of PCE removals in three experiments using lipid wastes as the substrate were found in the same level, 98-99 %. Sets of used 5,000 mg-used soybean oil/kg and 5,000 mg- oil wastes from Leo Food /kg had the best PCE removal efficiency of 99.46%. Although, used lard at 500 mg/kg has slightly lower efficiency (99.41%) than that of the above sets, it was considered interesting because of the least amount of material.

For further discussion, we have investigated the cost of waste-grade substrate used for each reactor as shown in table 4.3. The table also shows the content of carbon and hydrogen in each waste which was estimated based on the fact that major

component of used edible oil is linoleic acid which compose with C_{18} H_{32} O_2 and molasses consist of mainly 43.8% total sugar ($C_{12}H_{24}O_{12}$). It can be said from the table that used lard at 500 mg/kg-sludge provided same level of PCE dechlorination with lowest material cost comparing to the other substrates. Therefore it was chosen as best substrate to be applied in our next experiment.

Interestingly, dechlorination using carbohydrate substrate (molasses) in this experiment provided same level of removal efficiency as the ones using lipid substrates (three oil wastes). This result is quite consistent to the result of previous experiments using glucose and soybean oil which carbohydrate substrate (glucose) resulted in higher removal efficiency than the lipid one, soybean oil. The explanation of this could be that the easiness of degradation played important role to the dechlorination efficiency of food grade substrate. Therefore, glucose which has more simple structure provided higher dechlorination efficiency in the previous experiment. On the other hand, the amount of hydrogen atom in molecular structure has strong effect on the dechlorination efficiency of substrate with complex molecule such as molasses, used soybean oil and oil waste. For this reason, the used soybean oil and oil wastes which have larger amount of hydrogen atom in molecular structure presented higher removal efficiency than that of the molasses.

Table 4.3 Cost analysis and other information

Parameters	Molasses (mg/kg-sludge)		Used lard (mg/kg-sludge)		Used soybean oil (mg/kg-sludge)		Oil waste (mg/kg-sludge)	
	5,000	10,000	500	5,000	500	5,000	500	5,000
Cost ($*10^{-3}$ Baht/ reactor)	6.25	12.50	0.125	1.25	0.25	2.50	0.50	5.00
% PCE Removal	98.82 ± 0.84	97.47 ± 1.34	99.41 ± 0.65	98.13 ± 0.51	99.10 ± 0.69	99.46 ± 1.65	99.00 ± 0.26	99.46 ± 0.74
Estimated carbon content (mg-C/ mg-substrate)	0.4	0.4	0.77	0.77	0.77	0.77	0.77	0.77
Estimated hydrogen content (mg-H/ mg-substrate)	0.07	0.07	0.11	0.11	0.11	0.11	0.11	0.11

Moreover, it could be that the molasses might consist of some impurities that were toxic to bacteria in the system. This is reason why higher load of molasses addition can cause low PCE removal efficiency.

From this result, edible oil waste, especially used lard, can be reused in the remediation of PCE contaminated soil which enough high dechlorination efficiency and low material cost can be expected.

4.3.2 Production of PCE Intermediates, TCE and DCE

PCE intermediates (i.e. TCE and DCE), were detected using GC-ECD in order to confirm the PCE reductive dechlorination under anaerobic condition.

From figure 4.12, the highest TCE production was the 5,000 mg-oil waste/kg-sludge, next were 5,000 mg-used soybean oil/kg-sludge and 500 mg-Lard/ kg- sludge, respectively. Other set of reactors also produced TCE and the amount of TCE production seemed to be similarly using food grad as substrate. For DCE production, oil waste from Leo Food was the only type of carbon source which can produce DCE. However, the amount of DCE from this experiment was not so high (Figure 4.13).

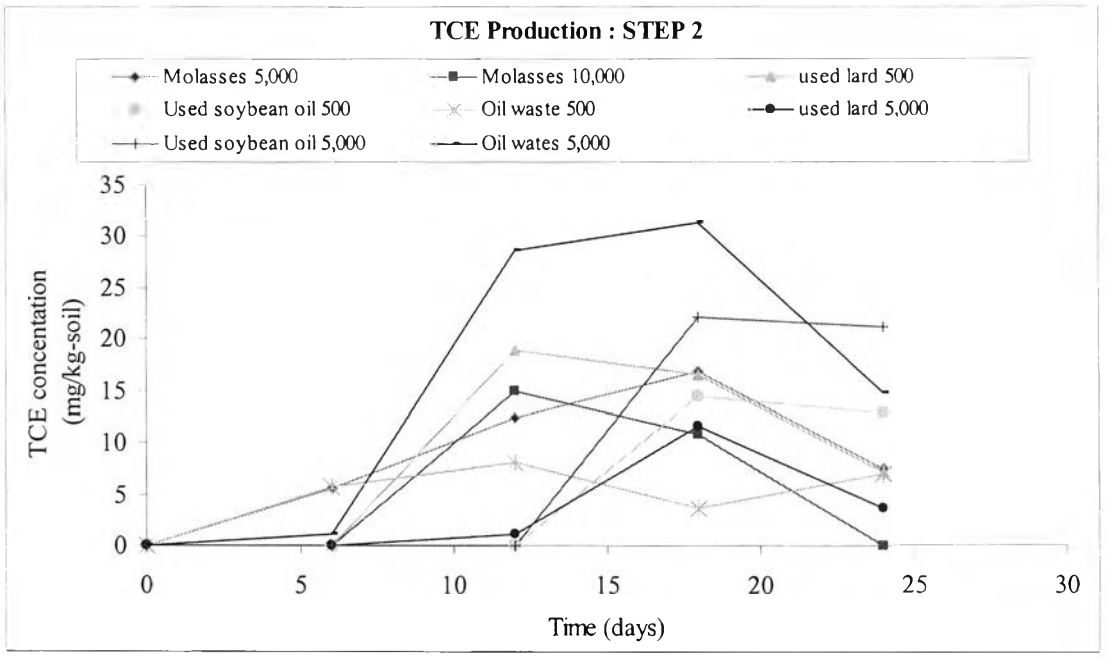

Figure 4.12 Total TCE production (mg/kg-soil) using wastes as carbon source.

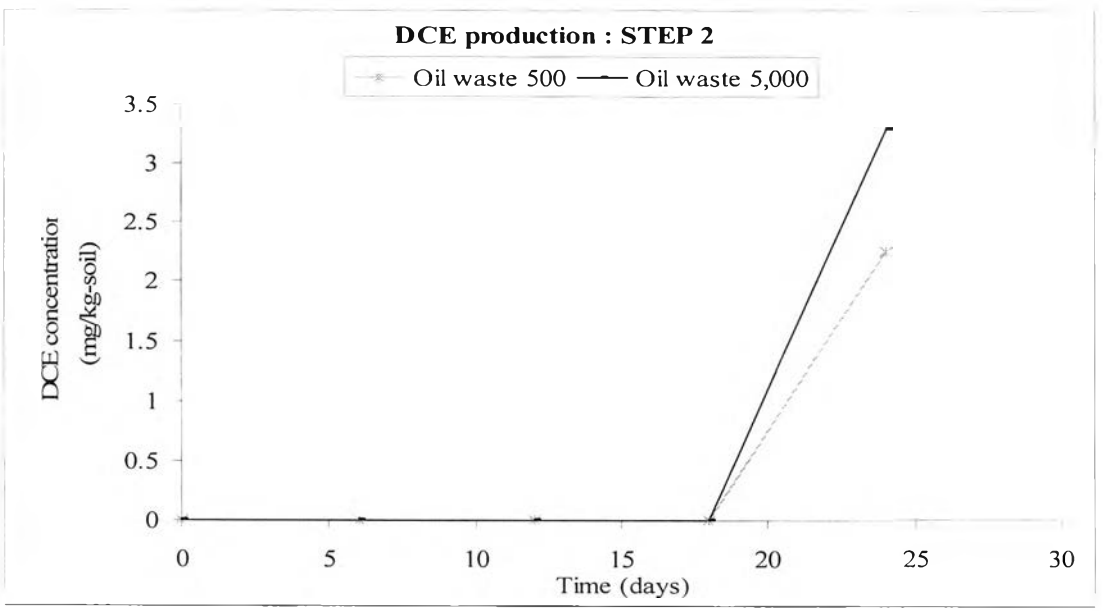

Figure 4.13 Total DCE production (mg/kg-soil) using wastes as carbon source.

Figure 4.13 showed total DCE that were produced in this experiment by oil waste from Leo Food industry. DCE was detected only from the case of oil waste from Leo Food the reason for this could be that the sludge and oil waste came from the same source which was Leo Food Industry, Chiang Mai. Therefore, bacteria in the sludge are more familiar with this Leo Food oil waste rather than other substrates. Consequently, respiratory process was then performed in higher speed and resulted in the higher production of DCE in the case of Leo Food oil waste.

It was noticed that other PCE intermediates i.e. vinyl chloride, ethene, ethane were not detected from this experiment. The explanation for this could be described as following. Contrary to food grade substrate, wastes have complex structures and lots of impurities, so they could become toxic and slow down the degradation at the same time of contributing carbon to the bacteria in unacclimated sludge. Therefore complete reductive dechlorination form PCE to TCE and other intermediates is far to reach with normal time period suggested by other studies using food grade substrate. However, because the time period for this study is limited, we could not perform the incubation for longer than 24 days.

4.3.3 Gas Production

Gas production was observed in order to confirm the respiratory process of anaerobic bacteria. The result of gas production was showed in figure 4.14. The best three gas production treatments were from used lard 500 mg/kg, used soybean oil

5,000 mg/kg and oil waste from Leo Food Industry 5,000 mg/kg, respectively. Gas was rapidly produced during the first six days from all wastes.

It is noticed that, the amount of gas production from wastes was less than that of food grade substrates in the previous experiment. As we have known that bacteria consumed carbon source from substrate under food digestion process to grow their cell and produce gas e.g. methane in the respiratory process as well as release hydrogen atom for the dechlorination. Comparing to the case of food grade substrate, it can be said that the hydrogen atoms released from waste substrates in this experiment were largely used for the dechlorination process rather than for producing methane gas.

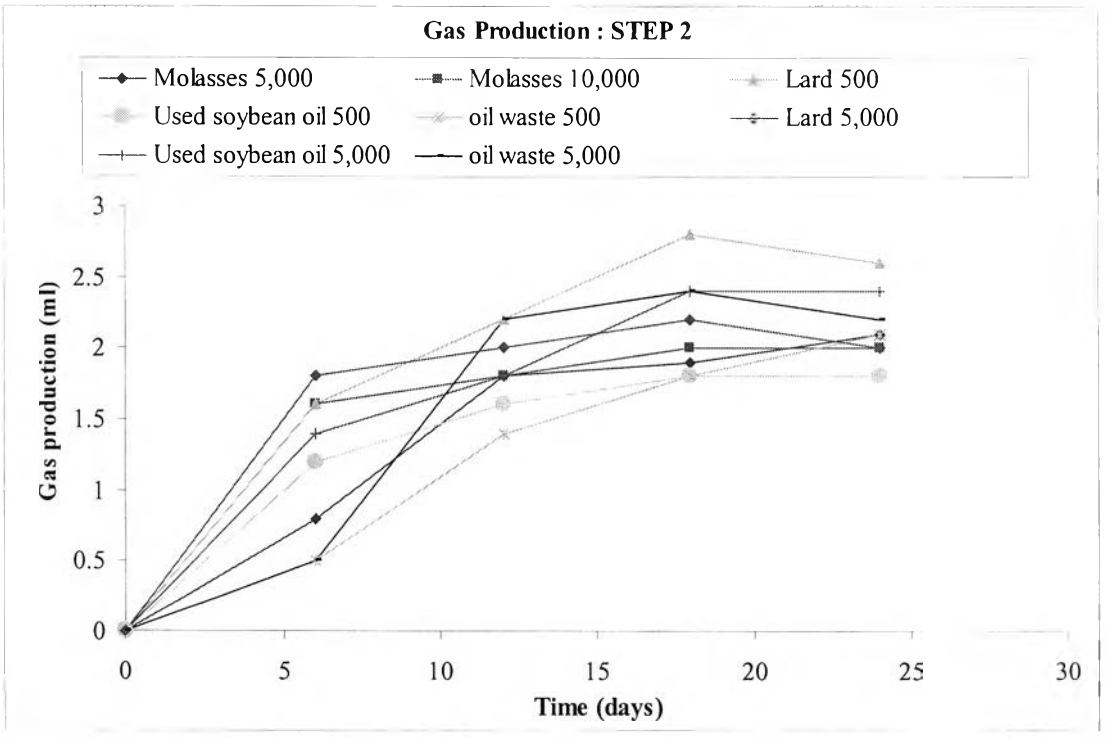

Figure 4.14 Gas production (ml) using waste as carbon source.

4.3.4 Chloride Production

Chloride production was also analyzed in order to confirm the progress of PCE reductive dechlorination process. The more chloride atoms were detected in the system, the higher PCE removal efficiency should be obtained.

Figure 4.15 showed chloride production in the soil slurry. All wastes continuously produced chloride in the system. Chloride rapidly increased during the first 6 day period. Three substrates that provided the highest chloride productions were the 500 mg-Lard/kg-sludge, 5,000 mg-used soybean oil/ kg-sludge and 5,000 mg-oil waste/ kg-sludge, respectively.

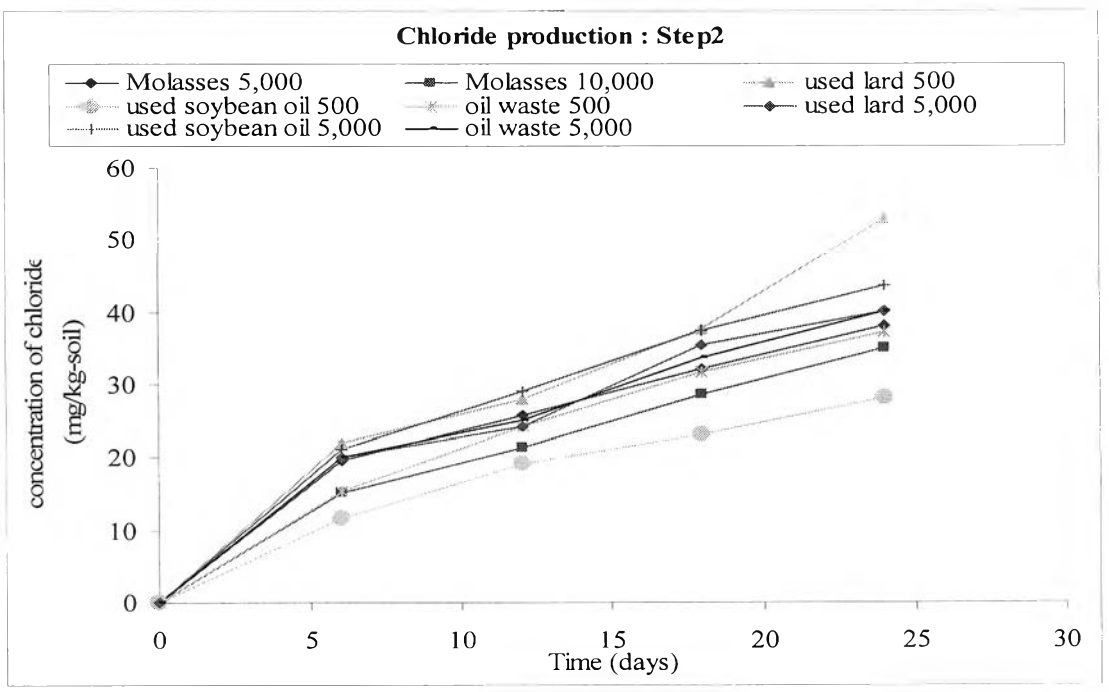

Figure 4.15 Chloride production using wastes as carbon source

4.4 Optimum concentration of PCE when using 500 mg-used lard/kg-sludge acts as electron donor for PCE reductive dechlorination

4.4.1 Decrease of PCE concentration

Used lard at 500 mg/kg-sludge was considered as the best waste that could acts as carbon source and electron donors in digestion and dechlorination processes for low cost bioremediation of the 100 mg-PCE/kg-soil contaminated soil which composed with 85.3 % sand: 11.6 % silt: 3.1 % clay. The removal efficiency was 99.41% which is the acceptable value comparing to the result of Doong and Wu, 1994. However, the concentration of PCE in the real situation can change all the time. Therefore, the ability of 500 mg/kg-sludge used lard for removal of the changing PCE should be further investigated. This part of experiments was done in order to clarify the ability of 500 mg/kg-sludge used lard for PCE reductive dechlorination.

Table 4.4 showed the PCE removal efficiency using 500 mg/kg used lard as electron donor fed batch. PCE at 100 mg/kg-soil was decreased the most, 99.10%. However, high removal efficiency was found for PCE concentration 150 mg/kg, 200 mg/kg and 250 mg/kg that were 88.70%, 87.93% and 80.68%, respectively.

Table 4.4 PCE removal efficiency using Lard 500 mg/kg as substrate

PCE concentration (mg-PCE/kg-soil)	C/Co (%)			Total PCE removal (%) (Triplicate)
	0	12	24	
1. 100	100 ± 1.90	12.32 ± 2.17	0.90 ± 2.14	99.10 ± 2.14
2. 150	100 ± 2.14	24.66 ± 1.33	11.30 ± 2.11	88.70 ± 2.11
3. 200	100 ± 2.26	26.04 ± 2.16	12.07 ± 1.98	87.93 ± 1.98
4. 250	100 ± 2.10	28.25 ± 2.06	19.32 ± 1.55	80.68 ± 1.55
5. 500	100 ± 1.86	86.00 ± 2.05	62.70 ± 2.12	37.30 ± 2.12

In the highest PCE concentration set (500 mg/kg-soil), the system was probably overloaded. The removal efficiency was only 37.305% which was the least. In the highly contaminated PCE, the chlorinated compound may become toxic to bacteria. The higher amount of PCE would kill bacteria in the system. When the amount of bacteria was deceasing, the degradation ability of decreased too and these brought to low PCE dechlorination efficiency. In this case the sludge to soil ratio could be adjusted in order to receive better removal efficiency. The increased quantity of sludge to soil ratio aimed to enhance the surviving bacteria in the system because a large amount of bacteria may be killed at the highly PCE concentration. When the surviving bacteria were increased and they can consume the organic carbon, the hydrogen atom in the system will be increased. However, it was necessary to study the appropriate sludge to soil ratio in lab scale before used in the real situation. The

extremely low removal efficiency in 500 mg/kg-soil could happen for this reason. For better understanding of this overload situation, further study is required.

Overall, PCE removal efficiency provided by lard was high when the concentrations of PCE were in the range of 100-250 mg/kg.

4.4.2 Production of PCE Intermediates, TCE and DCE

TCE produced from all sets of PCE varying experiments were shown in figure 4.16. Total amount of TCE production was consistent with the PCE removal efficiency using lard concentration 500 mg/kg-sludge as carbon source. For 100 mg-PCE/kg-soil set, largest TCE productions as well as highest PCE removal efficiency were observed. At the increasing PCE concentrations from 100 to 250 mg/kg soil, the TCE production was decreased, this TCE production was consistent with the decreased PCE removal.

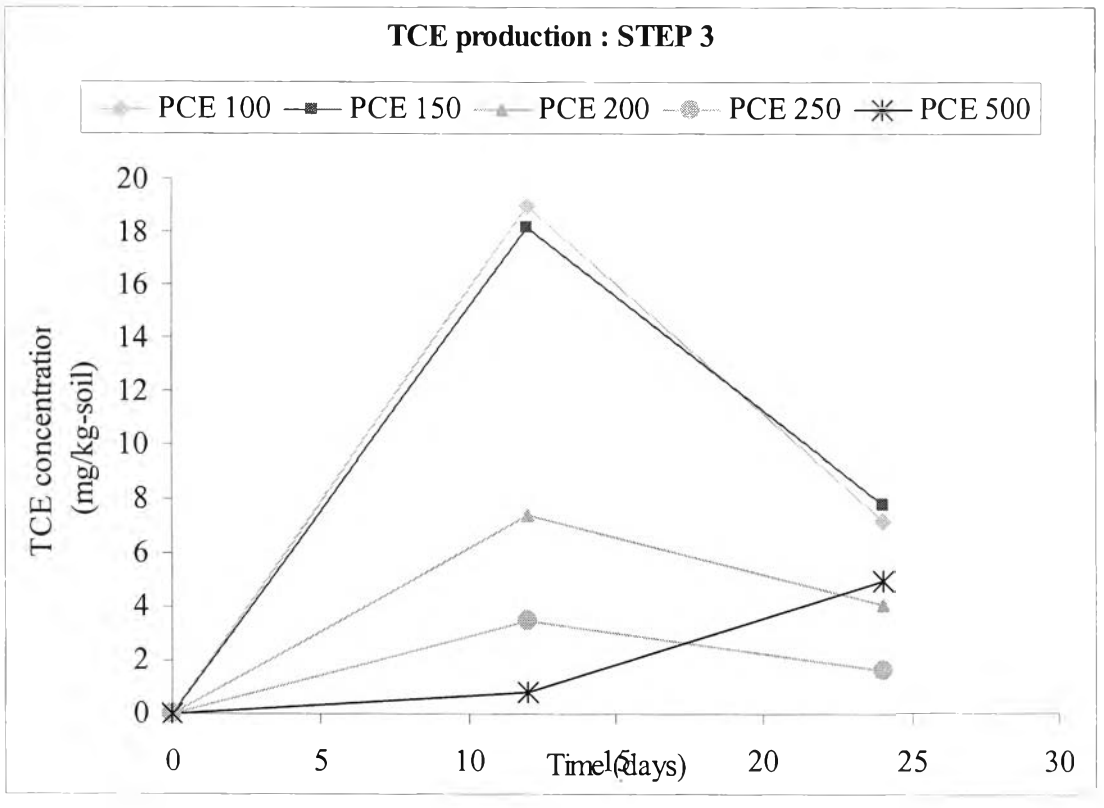

Figure 4.16 Total TCE productions (mg/kg-soil) when using the 500 mg-lard/kg-sludge as carbon source

However, the TCE production in 500 mg-PCE/kg-soil was increased. Although, PCE removal in this case was the least but this is the highest contaminated PCE therefore, only 37.3% PCE removal can induce the high TCE production. It is noticed that TCE production in this experiment was lower than that of the previous experiments. DCE and other intermediates i.e. vinyl chloride, ethene, ethane were not found in this experiment.

4.4.3 Total Gas Production

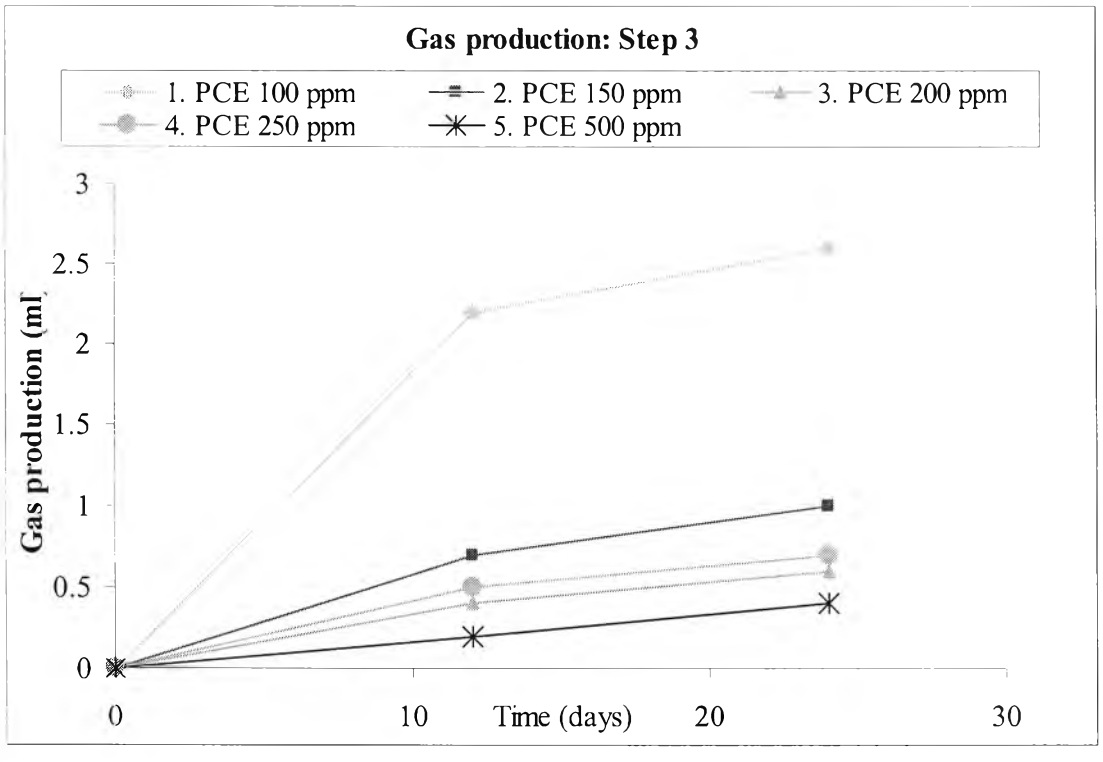

Figure 4.17 Total gas production (ml) using the used lard of 500 mg lard /kg-sludge as carbon source.

From figure 4.17, gas production of this experiment was not so high comparing to the previous ones. The reason for this is still unproved. It could be that the concentration of PCE increased from 100 to 500 mg/kg-soil, therefore bacteria need more time to acclimate with the high concentration of PCE. Bacteria used carbon source to grow their cell and then produce gas. The results indicated that high concentration of PCE decreased gas production, which probably due to the reduction of bacterial growth in this system.

4.4.4 Chloride Production

From Figure 4.18, chloride ion continuously increased within 24 days, when the PCE were dechlorinated in system.

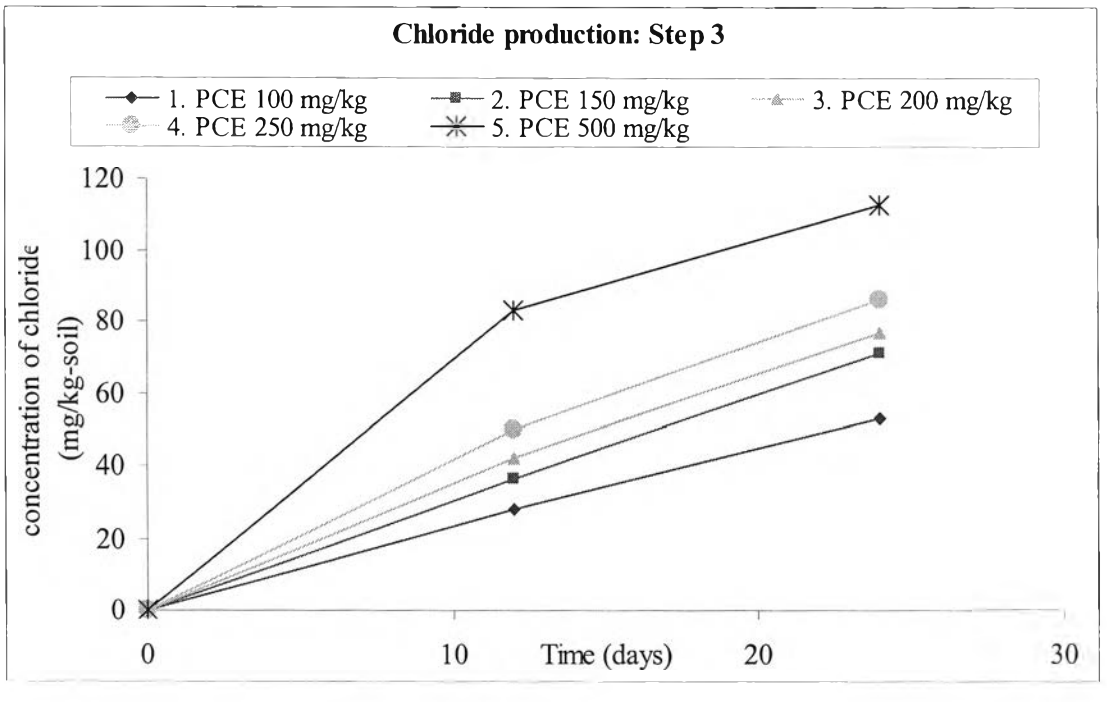

Figure 4.18 Chloride productions when using lard concentration 500 mg/kg-sludge as carbon source.

Hydrogen consumption was paralleled by chloride production, PCE degradation and intermediate formation. Accumulated TCE was found as the major product while DCE was likely the minor. All electrons derived from substrates oxidation were completely recovered in dechlorination product and biomass. Experiment with chloride was found in every step.