CHAPTER 4

RESULTS AND DISCUSSION

4.1 The role of carbon-sources supplementation

The data from each set of experiment was fitted by first-order equation in order to obtain comparable dechlorination rate constant.

The effect of yeast extract was investigated by comparing of two control sets, with and without yeast extract. It showed no significant difference between these two sets (see Figure 4.1) which provide rate constant at 0.0276 and 0.0217 day^{-1} for control set with and without yeast extract, respectively. Thus, it should be emphasized that the decreasing of HCB in this experiment does not depend on yeast extract. But the result of Chang et al's study (1997) showed that anaerobic degradation process favors yeast extract to mixed culture.

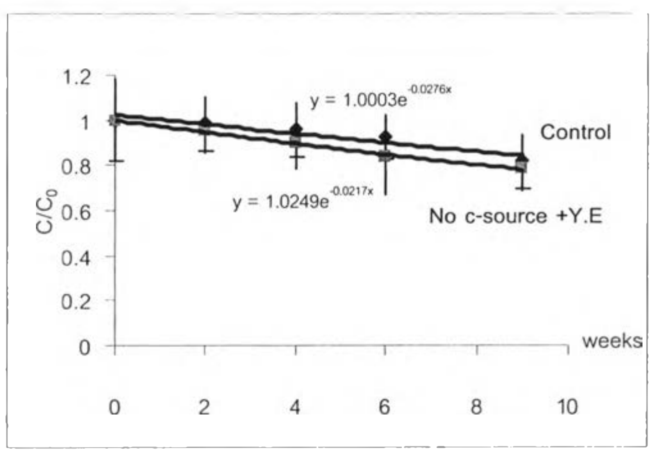


Figure 4.1 HCB concentration at different time comparing to the initial concentration (C/C_0). Control set with and without yeast extract.

The different results were obtained from experiment with different kind of substrates (Table 4.1). Comparing to control set, there's no significant decreasing of HCB concentration in control group with no carbon-source addition. Although, the organic content is quite high (11.35%) but these existing organic matter might recalcitrant for microorganisms in order to support microbial activity. This can be suggested that if we study on the ability of native microorganisms, it would be limited by rate at which natural organic matter can be hydrolyzed and transformed into bioavailable forms.

Table 4.1 Summary of the first-order rate constant and correlation efficient of HCB tested in different carbon source supplementation.

Sludge:sediment	Type of carbon source	Rate constant, k (d^{-1})
20:80	Glucose	0.066
50:50	Glucose	0.143
50:50	Lactate	0.114
50:50	Ethanol	0.074
50:50	Formate	0.065
50:50	Acetate/Butyrate/Propionate	0.044
50:50	No c-source addition	0.028
100:0	HCB enrichment culture	0.282 (Pavlostathis and Prytula 2000)
--	Estuarine sediment	0.026 (Susarla et al, 1997)

Comparing to the others' study, as the obtained rate constant of this study was in the range of 0.028 to 0.143 day^{-1}. Pavlostathis and Prytula (2000) showed dechlorination rate in HCB enrichment culture without sediment when supplemented with glucose and yeast extract was 0.282±0.011 day^{-1}. Susarla et al (1997) studied the dechlorination of HCB from Tsurumi River sediment without external carbon source addition, Japan was observed as 0.026 day^{-1} with the lag time of less than 1 day.

Glucose supplementation set showed the highest dechlorination rate among tested substrate in this study which k value of 0.143 day^{-1} as shown in Figure 4.2 when fitted with first order equation. The dechlorination has not been seen at first 2 weeks and then dramatically took place since the fourth week.

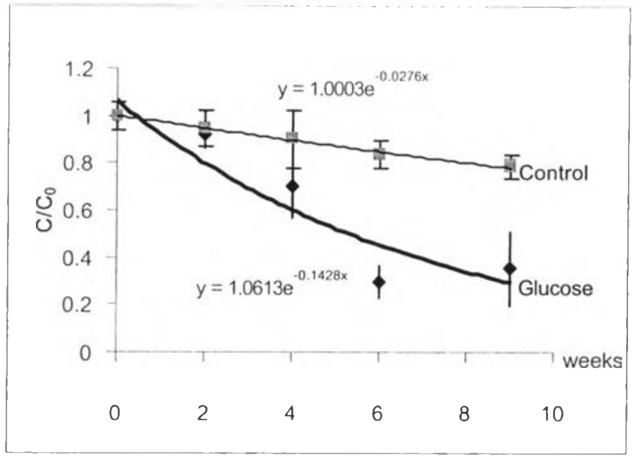


Figure 4.2 C/C_0 of HCB at different time of glucose supplementation set comparing to control set.

In anaerobic degradation process, glucose is transformed to volatile fatty acid (VFA), so it produced more H_2 to the system. Because of H_2 can act as electron donor, hence the more H_2 production, the more electrons for driving dechlorination activity. HCB acts as an electron acceptor, when obtained electron from H_2; the opportunity of releasing chloride ion and substitution of H_2 to HCB is increased. The existing of acidogen effected to the dechlorination of HCB when glucose was used as substrate. Acidogen may play an important role for dechlorination, but the internal mechanism is still unclear.

Supplementation with lactate provided high dechlorination rate (0.114 day^{-1}) as shown in Figure 4.3, but not as much as glucose supplementation. Lactate was one of the substrate that provided rapidly dechlorination, since acidogen metabolizes lactate to provide hydrogen and enhanced the production of methanogen, so higher dechlorination rate was observed. The observed dechlorination rate of ethanol supplementation in this study was 0.0735 day^{-1}, see in Figure 4.4.

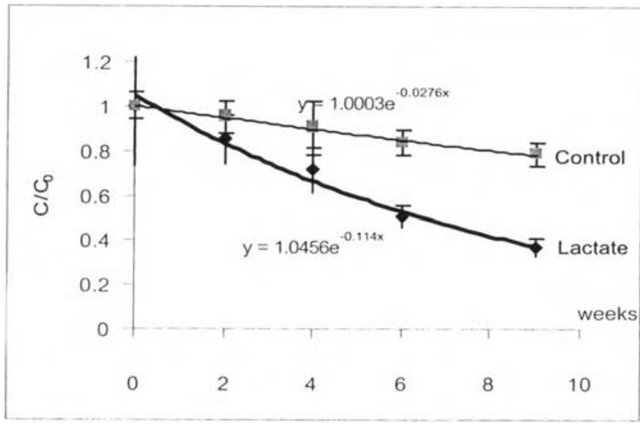


Figure 4.3 C/C_0 of HCB at different time of lactate supplementation set comparing to control set.

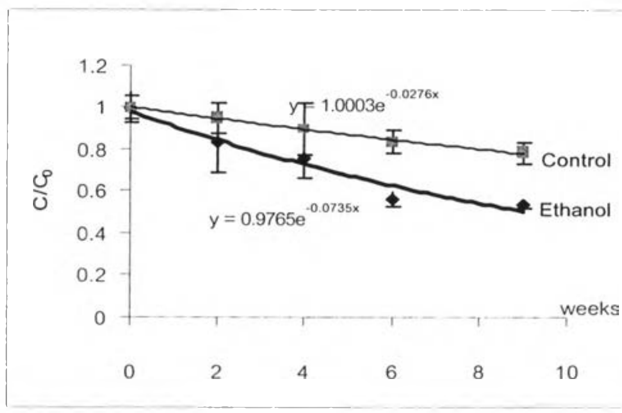


Figure 4.4 C/C_0 of HCB at different time of ethanol supplementation set comparing to control set.

Formate gave low dechlorination rate compared to other type of substrate. Formate may serve as unsuitable electron donor. 0.065 day^{-1} is the constant rate of dechlorination of formate supplementation set, as shown in Figure4.5.

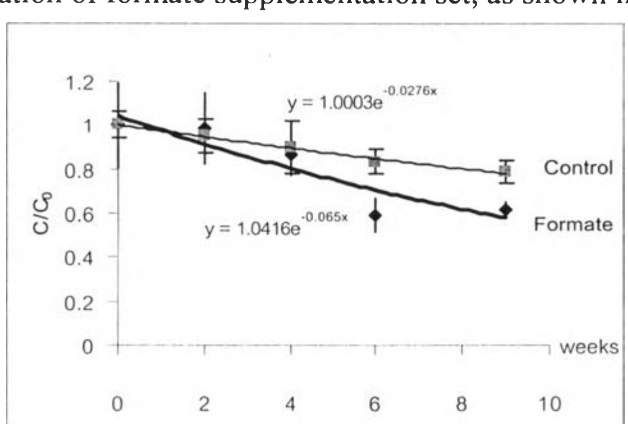


Figure 4.5 C/C_0 of HCB at different time of formate supplementation set comparing to control set.

No significant dechlorination activity was observed (Chang et al, 1996) when only of acetate was added to the system. Thus, the combination of acetate, butyrate and propionate was studied because they usually presence together during anaerobic degradation. So, it was expected to promote the dechlorination activity. But the observed dechlorination rate was quite low (0.0442 day^{-1}) comparing to other substrates, see in Figure 4.6. It revealed that the combination of these substrates may not served as electron donor or provides less amount of H_2.

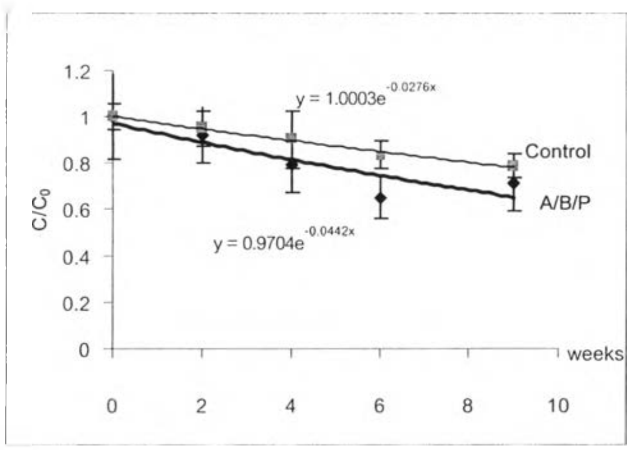


Figure 4.6 C/C_0 of HCB at different time of the combination of acetate, butyrate and propionate supplementation set comparing to control set.

The results of five experiments reported above corresponding to the previous works. Holliger et al (1992) showed that lactate, ethanol and hydrogen appeared to be the best substrates as giving the highest chloride production but in the presence of pyruvate, propionate, and formate, no dechlorination was observed. Peter et al (1994) found the production of chloride was highest with lactate, glucose and propionate but formate was the only electron donor tested which did not support the dechlorination of 1,2,4-TCB which was the intermediate of HCB dechlorination.

The effect of carbon source was also determined in less sludge amount to sediment as shown in Figure 4.7. Glucose was selected to use as the studied substrate, because of the highest dechlorination efficiency. The result revealed that even supplement with the best carbon source, but using less amount of biomass, the dechlorination efficiency is low. The observed rate constant was 0.0359 and 0.0663 day^{-1} for 20:80 sludge to sediment ratio without and with glucose supplementation.

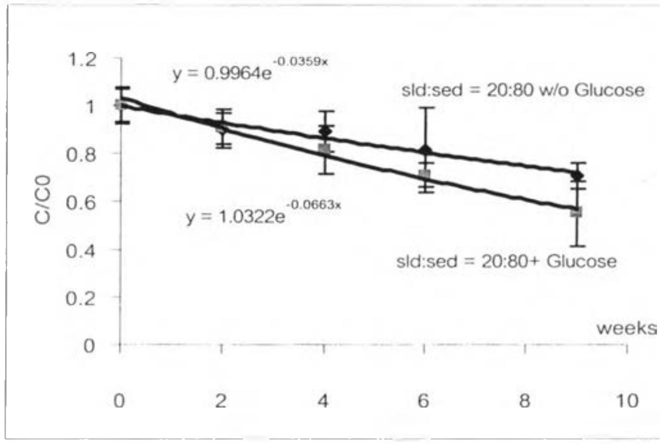
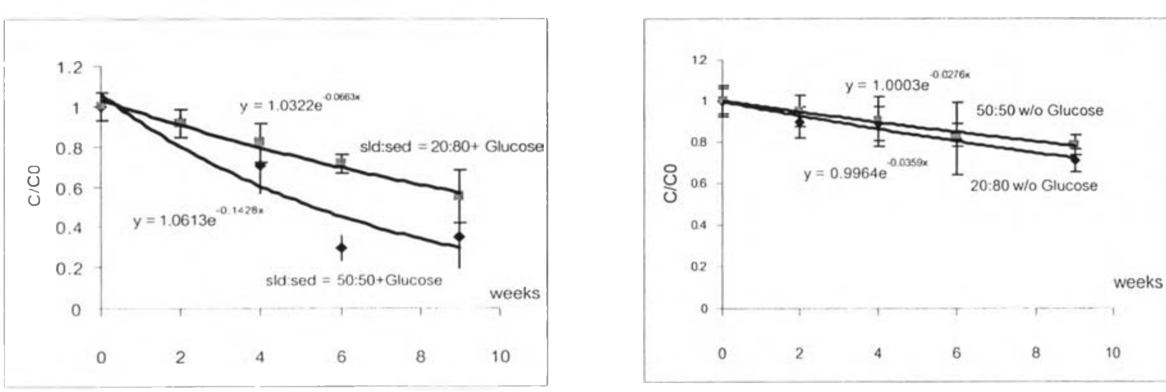


Figure 4.7 C/C_0 of HCB at different time of 20:80 (sludge to sediment ratio) with and without glucose supplementation set comparing to control set.

4.2 The role of sludge to sediment ratio

Figure 4.8 (a) showed the different rate of dechlorination obviously that the more sludge content, the higher dechlorination rate in the presence of substrate. Rate constant of 20:80 and 50:50 sludge to sediment ratio was 0.0663 and 0.1428 day^{-1}, respectively. If HCB is toxic to microorganisms, when larger quantity of sludge was added to degrade this substance, the opportunity of sludge to expose toxic is less than adding smaller sludge quantity. On the other hand, less dose of HCB contacts to microorganism when larger content of sludge was added.



(a) (b)

Figure 4.8 C/C_0 of HCB at different time of sludge to sediment ratio at 50:50 and 20:80 with (a) and without (b) glucose supplementation.

The result of this study related with Pavlostathis and Prytula's study (2000) that HCB dechlorination rate was almost directly proportional to the initial biomass concentration. For example, 3 diluted culture series as compared to that of the in diluted culture was 0.7, 0.56 and 0.30 corresponding to the three initial inoculum levels of 75, 50 and 25%, respectively.

The decreasing of HCB of different sludge to sediment quantity showed very similar manner when there was no substrate addition (Figure 4.8 (b)). It may be said that unacclimated sludge needs electron donor in order to enhance the dechlorination activity.

4.3 The combined role of c-source supplementation and sludge to sediment ratio

Figure 4.9 showed the combined role of carbon-source supplementation and sludge quantity base on dechlorination rates.

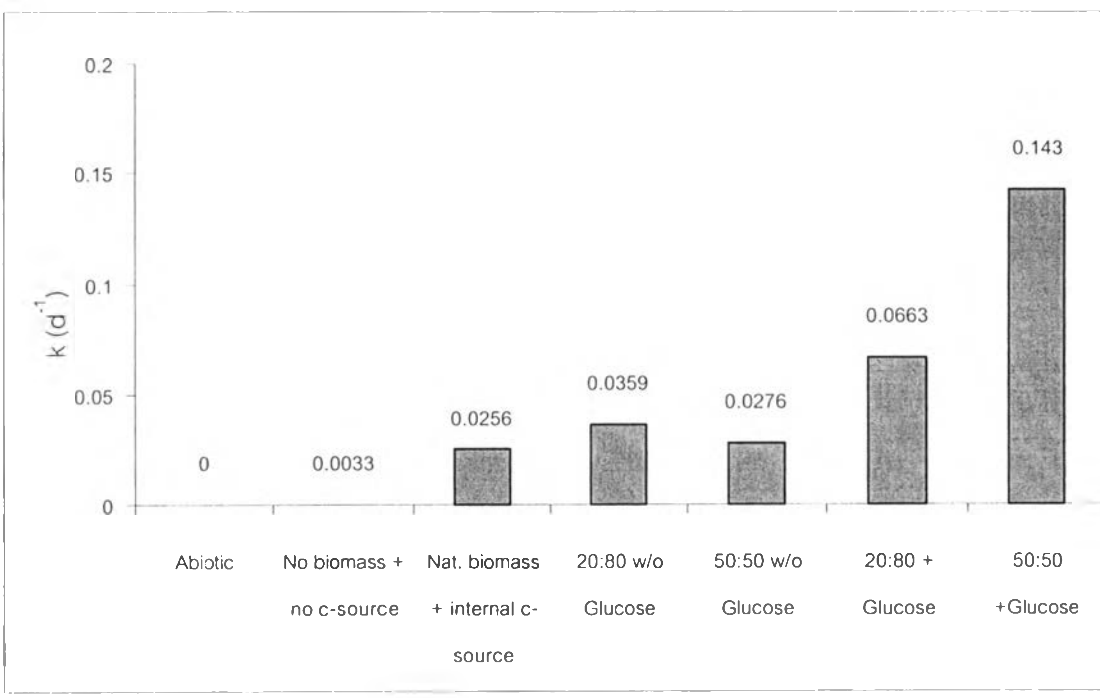


Figure 4.9 k value (dechlorination rate) of various experiment

The comparable dechlorination rate due to the effect of c-source supplementation and sludge to sediment ratio could be observed in this study. Rate of dechlorination was 0 day^{-1} under abiotic condition and 0.003 day^{-1} in soil under aerobic degradation (Howard et al, 1991). In estuarine sediment containing natural biomass with its natural c-source, dechlorination rate was 0.0256 day^{-1} (Susarla et al, 1997).

The effect of c-source could be seen by consider dechlorination rate of 0.0359 day^{-1} and 0.0663 day^{-1} from the experiment at 20:80 sludge to sediment ratio with and without glucose, respectively and 0.0276 $day^{-1}$and 0.143 day^{-1} from the experiment at 50:50 sludge to sediment ratio with and without glucose, respectively. On the other hand, k value was increased 5.18 times higher when supplemented with glucose, but it was insignificantly increased in case of 20:80 sludge to sediment ratio.

The effect of sludge to sediment ratio could be seen in increasing k value from 0.0663 day^{-1} to 0.143 $day^{-1}$with sludge to sediment ratio from 20:80 to 50:50 and supplemented with glucose. It was about 2.16 times increasing in k value when higher sludge quantity was performed with glucose addition but no significant increasing in k value was observed when there was c-source supplementation even higher sludge to sediment ratio was performed.

K value from the combined role of both c-source supplementation and higher sludge to sediment ratio (glucose supplementation at 50:50 sludge to sediment ratio) was about 5.59 times increasing comparing to dechlorination rate under natural condition.

The results show in Figure 4.9 revealed that external c-source and sludge quantity were considered as important factor for HCB dechlorination, which were able to accelerate the dechlorination rate in contaminated sediment.

4.4 Total gas and methane production

The amount of gas produced was recorded at designated time by determination from the elevation of septum, shown in Figure 4.10. This septum was pushed up by gas produced from microbial activity. However, it may not represent the actual gas production because of some limitation. For example, the friction between septum and inner surface of syringe resulted in less total gas detection.

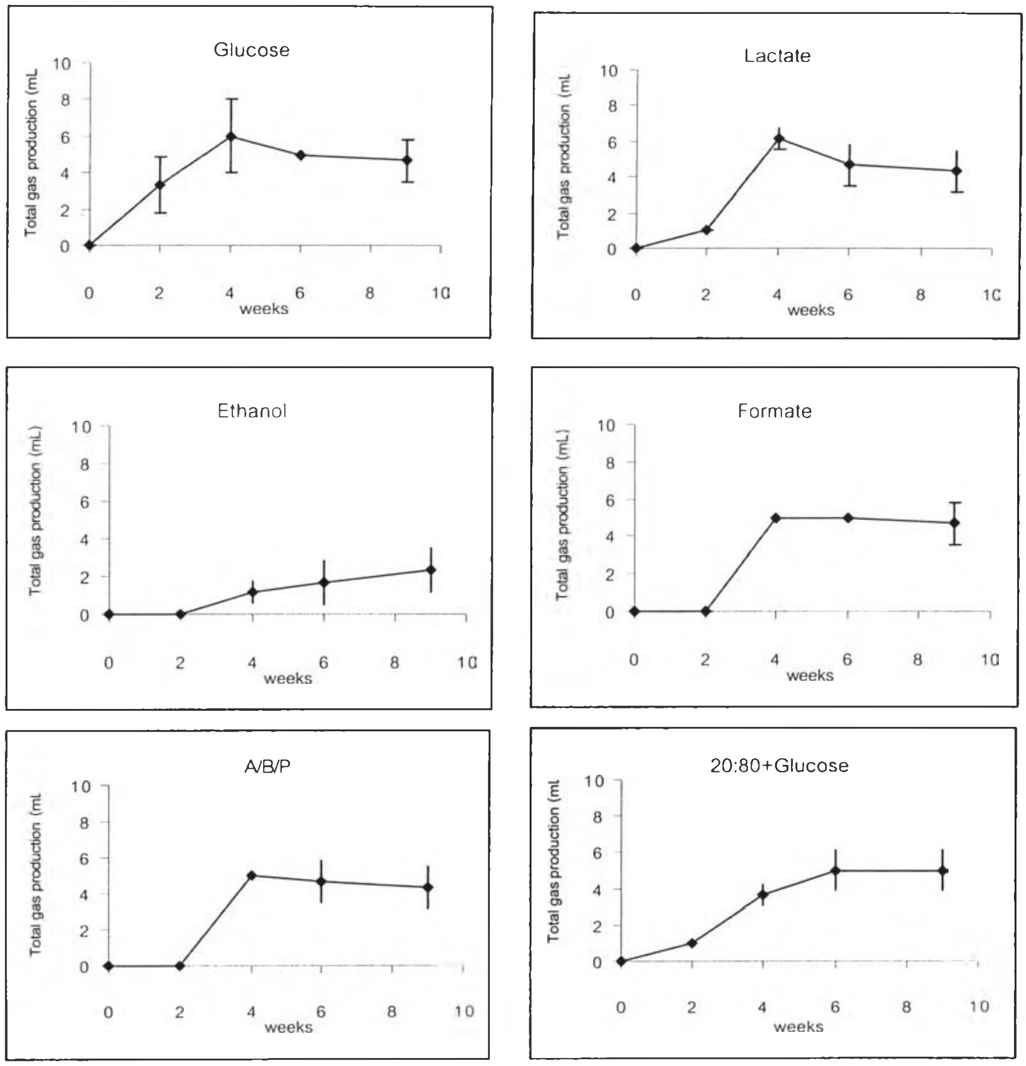


Figure 4.10 Total gas production (ml) from each set of experiment.

Gas production during first 2 weeks of some experiments may be undetectable due to friction loss, and only two sets of experiment produced gas at fourth week. Ethanol supplementation set seemed to produce the least. Most of gas production was almost quite consistent after the fourth week.

Microbial activity in the present of c-source increased much more rapidly, as methane production was observed (Prytula and Pavlostathis, 1996). The highest methane production was obtained from the experiment of glucose supplementation set. (Figure 4.11). Methane percent was nearly the same from the other sets of experiment. There was very less methane concentration existing in the system. This can be explained as the circulation of methane consumption by microorganisms existing in the system, where no change of environment in the test-tube. Nevertheless, it cannot be said that there was the relationship between percent of HCB removal and produced methane concentration.

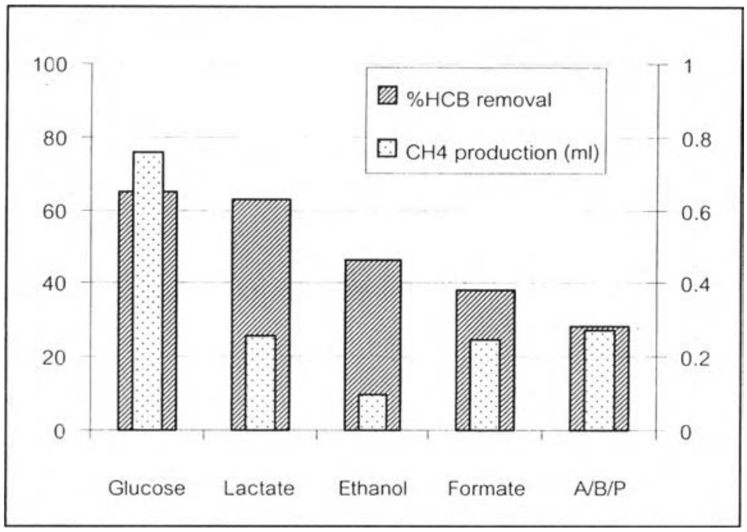


Figure 4.11 %HCB removal couple with methane production (ml) at final set of experimental time.

Although, it cannot be concluded that HCB dechlorination occurs under sulfate- reducing, nitrate-reducing or methanogenic reducing condition. Because sulfate was added in nutrient solution, so sulfate-reducing condition may occur. But nitrate-reducing condition was never happen because nitrate was not added in nutrient solution. The production of CH_4 revealed that methanogens do exist in this reductive dechlorination.

4.5 The recovery of HCB from slurry sediment

The result of HCB recovery from this experiment showed very less percent recovery, see Table 4.2. LeBoeuf E. J has been working on the understanding of how contaminants interact with organic matter in soils and sediments. As very high organic matter content in this study was observed (11.35%). Organic contaminants such as chlorinated hydrocarbon are attracted to organic matter present in soils. Many clues are still unknown with the respect to controlling behavior of contaminant molecule work their way into organic matter. For example, how strongly the contaminant will be bound to the organic matter in soil, how long it will take to diffuse into soil or how fast it will be released back into the environment. He observed that when organic matter is in a glassy state, it has multiple pores and holes and thus poses a higher sorption capacity than when it is in a rubbery state. It was expected that the contaminants may leach from soils do not reach significant levels.

Table 4.2 Recovery efficiency of HCB using hexane for extraction from different matrix.

Matrix	% Recovery
Water	70-80
Sediment	15-35

However, extraction of wet sediment achieving nearly 100% had been done via sequential solvent extraction. But this method took over a week to perform and would be inappropriate for frequent sample analysis (Prytula and Pavlostathis, 1996).

4.6 Possible mechanisms

The actual mechanism which cause the decreasing of HCB in this experiment can not be exactly described by any known mechanism. There are 4 proposed mechanisms that possible to take place during the experiment as shown in Figure 4.12.

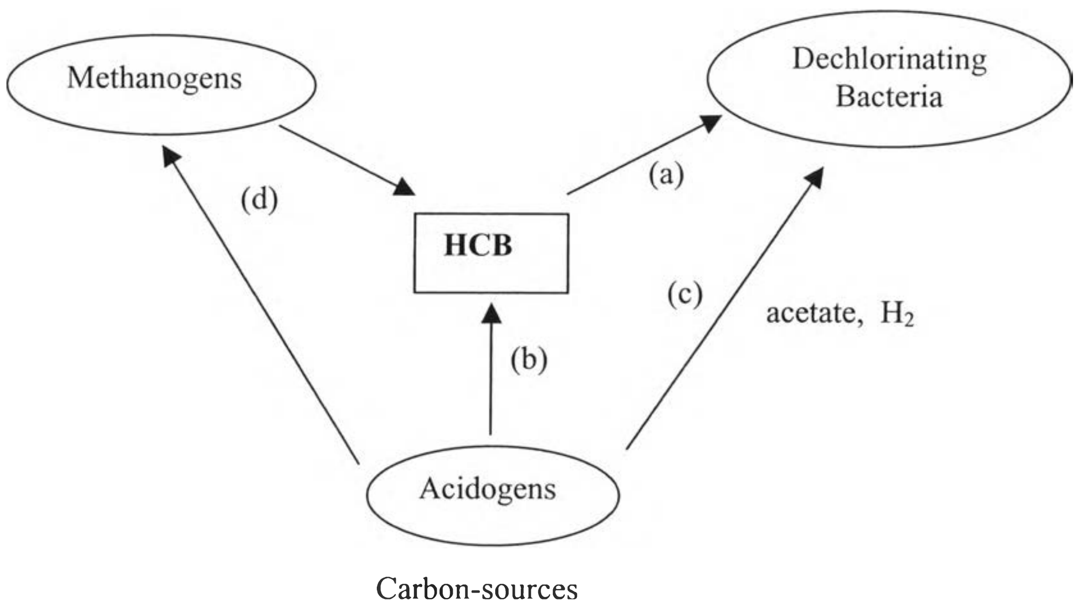


Figure 4.12 Possible mechanisms which can cause the decreasing of HCB

a) The presence of HCB may stimulate the activity of dechlorinating bacteria directly.
b) Carbon-sources serve as substrate for acidogens and corresponding to assist dechlorination activity and then intermediates were produced.
c) By product from degradation by acideogens such as acetate and H_2 stimulate HCB dechlorinating bacteria activity.
d) The decreasing of HCB caused by dechlorination by methanogens or may be adsorbed to methanogens' cell.

However, mechanism (b) was considered as the most possible one due to the results from this study. Supplementation with glucose, lactate and ethanol provided the first three highest rate of dechlorination among tested substrates. As acidogen involves in the first step of anaerobic degradation (section 2.2) for breaking down complex organic materials to be simple molecule such as glucose was transformed to VFA. Lactate and ethanol was metabolized to other compounds by acidogen. This implied that the reaction which acidogen has involved could promote the dechlorination activity.

The dechlorination rate of formate and the combination of acetate, butyrate and propionate supplementation experiment were less than that of glucose, lactate and ethanol. Hence, mechanism (d) and (c) may not be considered as the main dechorination process. Because formate and acetate involve both mechanism (d) and (c), which they were metabolized to methane and carbon dioxide by methanogen, as represented by mechanism (d) or serve as electron donors for dechorination with HCB act as an electron acceptor, as represented by mechanism (c).

Mechanism (a) was also difficult or rarely to occur because sludge seed has never been exposed to chlorinated compounds as it was obtained from wastewater treatment plant of soft drink manufacturing. And no acclimation period prior the experiment was performed. Therefore, it was quite impossible that dechlorinating bacteria would be developed that soon within two months.