อากาศในตะกอนก้นลำน้ำตามธรรมชาติได้ซึ่งสอดคล้องกับผลการวิเคราะห์ตะกอนที่เก็บจากคลองหัวลำภูซึ่งพบ 1,3,5-ไตรคลอโรเบนซินร่วมอยู่ด้วย ซึ่งหากเปรียบเทียบกับผลการศึกษาในที่ (in situ) ของงานวิจัยหลายชิ้นจะพบได้ว่าจุลชีพในตะกอนจากคลองหัวลำภูมีความไวปฏิกิริยาต่อการย่อยสลายเฮกซะคลอโรเบนซินสูงกว่ามาก เช่น Beurskens และคณะ (1994) ตรวจสอบตะกอนก้นทะเลสาบ Ketelmeer ซึ่งเป็นพื้นที่ตกตะตอนของแม่น้ำ Rhine และพบว่าร้อยละ 80 ของเฮกซะคลอโรเบนซินที่ปนเปื้อนในตะกอนถูกย่อยสลายไปในช่วงเวลา 20 ปี ในขณะที่ Zhao และคณะ (2003) พบค่าครึ่งชีวิตของเฮกซะคลอโรเบนซินในตะกอนเท่ากับ 1.7 ปี

4.3 การลดคลอรีนของโพลีคลอริเนเต็ดไบฟีนิล

4.3.1 ผลการศึกษาในน้ำตะกอน

4.3.1.1 ความสามารถในการย่อยสลายของจุลชีพแบบไร้อากาศ

ในงานวิจัยครั้งนี้คณะทำงานเลือกที่จะทดสอบความสามารถในการย่อยสลายของจุลชีพด้วย 2,3,4- และ 3,4,5-ไตรคลอโรไบฟีนิลก่อนเพราะทั้งสองคอนเจนเนอร์นี้ไม่เป็นส่วนผสมของสารประ กอบพีซีบีที่มีจำหน่ายทางการค้าเช่น Aroclor 1242, Aroclor 1254 และ Aroclor 1260 การกระทำในลักษณะนี้จะทำให้ไม่เกิดข้อผิดพลาดจากการปนเปื้อนเดิมในตะกอน ผลการลดคลอรีนโดยน้ำตะกอนจากคลองหัวลำภูได้แสดงไว้ในตารางที่ 4.11 ซึ่งพบว่า 2 มิลลิกรัมต่อลิตร 2,3,4-ไตรคลอโรไบฟีนิลสามารถถูกย่อยสลายได้โดยคลอรีนอะตอมที่ถูกดึงออกจากโมเลกุลจะเป็นคลอรีนอะตอมในตำแหน่งกลางเหมือนเช่นในกรณีของ 1,2,3,5-เตตระคลอโรเบนซิน(แต่ใช้เวลาในการย่อยสลายนานกว่าเฮกซะคลอโรเบนซิน) และการศึกษาการลดคลอรีนของไตรคลอโรไบฟีนิลโดย Williams (1994) การทำซ้ำที่ Sites A3 และ A4 ให้ผลใกล้เคียงกัน แต่ถ้าใช้น้ำตะกอนจาก Site E1 และ E2 จะพบว่าต้องใช้เวลานานมากในการย่อยสลาย ชี้ให้เห็นว่า 2,3,4-ไตรคลอโรไบฟีนิลสามารถย่อยสลายทางชีวภาพได้แต่อาจใช้เวลานาน ในทำนองกลับกันพบว่า 3,4,5-ไตรคลอโรไบฟีนิลย่อยสลายได้ยากมาก มีเพียงน้ำตะกอนจาก Sites A1, A3#1 และ A4#1 เท่านั้นที่สามารถเปลี่ยน 3,4,5-ไตรคลอโรไบฟีนิลให้เป็น 3,5-ไดคลอโรไบฟีนิลได้ และเช่นเดียวกันคลอรีนอะตอมในตำแหน่งกลางจะถูกแยกออกจากโมเลกุลของไบฟีนิลก่อน

จากผลการทดสอบขั้นต้นพบว่าการย่อยสลายพีซีบีทางชีวภาพแบบไร้อากาศมีความเป็นไปได้ คณะทำงานจึงศึกษาการลดคลอรีนกับคอนเจนเนอร์ที่มีอยู่ในพีซีบีที่จำหน่ายทางการค้าโดยทำการเลือกมา 19 คอนเจนเนอร์ที่มีคลอรีนเป็นองค์ประกอบตั้งแต่ 2 ถึง 6 อะตอมดังแสดงในตารางที่ 4.12 โดยใช้น้ำตะกอนจาก Sites A3 ผสมกับ Site A4 เพื่อเพิ่มความ

ตารางที่ 4.11 การลดคลอรีนของ 2,3,4- และ 3,4,5-ไตรคลอโรไบฟีนิลในน้ำตะกอน

Congener	Site	Lag Phase (weeks)	Complete Dechlorination (weeks)	Products
2,3,4-CBp	A1	2	12	2,4-CBp
	A2	2	10	2,4-CBp (major) & 2,3-CBp (minor)
	A3#1	4	>8	2,4-CBp
	A3#2	2	12	2,4-CBp (major) & 2,3-CBp (minor)
	A4#1	2	>8	2,4-CBp
	A4#2	4	10	2,4-CBp
	A5	2	10	2,4-CBp
	A6	2	>18	2,4-CBp
	A7	2	12	2,4-CBp
	A8	2	12	2,4-CBp
	A9	2	12	2,4-CBp
	A10	2	12	2,4-CBp
	E1	10	>16	2,4-CBp
	E2	14	>18	2,4-CBp
3,4,5-CBp	A1	56	>12	3,5-CBp
	A2	ND	ND	ND
	A3#1	154	>28	3,5-CBp
	A3#2	ND	ND	ND
	A4#1	126	>28	3,5-CBp
	A4#2	ND	ND	ND
	A5	ND	ND	ND
	A6	ND	ND	ND
	A7	ND	ND	ND
	A8	ND	ND	ND
	A9	ND	ND	ND
	A10	ND	ND	ND

หมายเหตุ: -ND คือตรวจไม่พบ/ไม่เกิดการลดคลอรีน

ตารางที่ 4.12 สัดส่วนของคอนเจนเนอร์ที่ศึกษาใน Aroclor

Congener	% in Aroclor 1242	% in Aroclor 1254	% in Aroclor 1260
2,2'-CBp	3.01	-	-
2,3'-CBp	1.38	-	-
2,4'-CBp	7.65	-	-
4,4'-CBp	1.51	-	-
2,5,2'-CBp	6.28	0.41	-
2,5,4'-CBp	4.59	0.22	0.05
2,4,4'-CBp	6.52	0.25	0.05
3,4,2'-CBp	4.79	0.14	-
2,4,2',5'-CBp	3.60	1.64	-
2,3,2',5'-CBp	3.20	-	-
2,5,3',4'-CBp	3.89	3.21	0.09
2,5,2',5'-CBp	-	-	-
2,3,4,4'-CBp	1.33	0.54	-
2,4,5,3',4'-CBp	1.62	6.39	0.57
2,3,6,3',4'-CBp	1.53	5.85	1.90
2,3,6,2',4',5'-CBp	0.63	2.21	7.83
2,4,5,2',4',5'-CBp	0.68	4.26	10.80
2,3,4,2',3',6'-CBp	0.30	1.98	3.69
2,3,4,2',4',5'-CBp	-	0.77	1.55

หลากหลายทางจุลชีววิทยา ผลการทดลองพบว่ามีเพียง 4 คอนเจนเนอร์คือ 2,4,4'-, 2,4,2',5'-, 2,3,2',5'- และ 2,3,4,4'-CBps เท่านั้นที่สามารถถูกลดคลอรีนได้โดยมีระยะเวลาพักตัว (Lag Phase) ที่ 18, 18, 8 และ 6 สัปดาห์ และได้ผลิตภัณฑ์คือ 2,4-, 2,4,2'-, 2,3,2'- และ 2,4,4'-CBps ตามลำดับ แต่ทั้ง 4 คอนเจนเนอร์ที่ความเข้มข้น 2 มิลลิกรัมต่อลิตรไม่สามารถถูกย่อยสลายได้หมดในช่วงเวลาที่ศึกษาคือ 18 สัปดาห์ดังแสดงในตารางที่ 4.13 เป็นที่น่าสังเกตว่าคลอรีนอะตอมในตำแหน่งเมตาจะถูกดึงออกจากวงไบฟินิลได้ง่ายกว่าตำแหน่งพาราเหมือนงานวิจัยของ Fish และ Principe (1994) และไม่เกิดการลดคลอรีนในตำแหน่งออโธเลยเหมือนเช่นในงานวิจัยของ William (1994), Mavoungou และคณะ (1991), Rhee และคณะ (1993) และ Natarajan และคณะ (1996) เพื่อเป็นการยืนยันผลความคงทนต่อการย่อยสลายของพีซีบี คณะทำงานได้ทดสอบกับน้ำตะกอนจากคลองข้าง ถ. ตำหรุ-บางพลี สมุทรปราการ (Sites E1 และ E2) ที่รับน้ำทิ้งจากร้านขายของเก่าซึ่งเชื่อว่ามีการล้างภาชนะที่น่าจะปนเปื้อนพีซีบี ผลพบว่าน้ำตะกอนจากทั้งสองจุดไม่สามารถลดคลอรีนของพีซีบีทั้ง 4 คอนเจนเนอร์ที่ทดสอบได้

ตารางที่ 4.13 การลดคลอรีนของพีซีบีคอนเจนเนอร์ต่างๆด้วยน้ำตะกอน

Site	Congener	Lag Phase (weeks)	Product(s)	Complete Dechlorination (weeks)
A3+A4	2,2'-CBp	ND	ND	ND
	2,3'-CBp	ND	ND	ND
	2,4'-CBp	ND	ND	ND
	4,4'-CBp	ND	ND	ND
	2,5,2'-CBp	ND	ND	ND
	2,5,4'-CBp	ND	ND	ND
	2,4,4'-CBp	18	2,4-CBp	>18
	3,4,2'-CBp	ND	ND	ND
	2,4,2',5'-CBp	18	2,4,2'-CBp	>18
	2,3,2',5'-CBp	8	2,3,2'-CBp	>18
	2,5,3',4'-CBp	ND	ND	ND
	2,5,2',5'-CBp	ND	ND	ND
	2,3,4,4'-CBp	6	2,4,4'-CBp	>18
	2,4,5,3',4'-CBp	ND	ND	ND
	2,3,6,3',4'-CBp	ND	ND	ND
	2,3,6,2',4',5'-CBp	ND	ND	ND
	2,4,5,2',4',5'-CBp	ND	ND	ND
	2,3,4,2',3',6'-CBp	ND	ND	ND
	2,3,4,2',4',5'-CBp	ND	ND	ND
E1	2,5,2'-CBp	ND	ND	ND
	2,5,4'-CBp	ND	ND	ND
	2,3,2',5'-CBp	ND	ND	ND
	2,4,5,3',4'-CBp	ND	ND	ND
E2	2,5,2'-CBp	ND	ND	ND
	2,5,4'-CBp	ND	ND	ND
	2,3,2',5'-CBp	ND	ND	ND
	2,4,5,3',4'-CBp	ND	ND	ND

หมายเหตุ: -ND คือตรวจไม่พบ/ไม่เกิดการลดคลอรีน

4.3.1.2 ผลของสารอาหาร

เนื่องจากพบว่าพีซีบีมีความคงทนสูงกว่าเฮกซะคลอโรเบนซินมาก คณะทำงานจึงได้ศึกษาหาตัวแปรที่จะช่วยส่งเสริมการลดคลอรีนของพีซีบีโดยใช้ 2,3,4-ไตรคลอโรไบฟีนิลเป็นสารเป้าหมายเนื่องจากพบว่าสามารถถูกย่อยสลายได้ในเวลาที่ไม่นานมากนัก น้ำตะกอนที่ใช้ทดสอบมาจาก Sites E1 และ E2 ผลที่ได้แสดงไว้ในตารางที่ 4.14 ซึ่งพบว่าการเติมสารอาหารทั้งในรูปของแลคเทตและไพรูเวตมีผลที่เด่นชัดต่อการลดคลอรีนของ 2,3,4-ไตรคลอโรไบฟีนิลมากกว่าในกรณีของเฮกซะคลอโรเบนซิน กล่าวคือสามารถช่วยให้ 2,3,4-ไตรคลอโรไบฟีนิลย่อยสลายได้เร็วขึ้น ส่วนการเติมอะซิเตทมีแนวโน้มที่จะไปรบกวนการทำงานของจุลชีพกลุ่มที่ลดคลอรีน ส่วนสารสกัดยีสต์ไม่มีผลที่เด่นชัดต่อการลดคลอรีน อย่างไรก็ดีถึงจะมีการป้อนสารอาหารและธาตุอาหารเสริมเพิ่มให้มากเกินพอ 2,3,4-ไตรคลอโรไบฟีนิลที่ 2 มิลลิกรัมต่อลิตรก็ไม่สามารถถูกย่อยสลายได้หมดในช่วงระยะเวลาที่ศึกษา ผลของสารอาหารต่อการลดคลอรีนของพีซีบีที่ได้นี้สอดคล้องกับงานวิจัยของ Nies และ Vogel (1990) ซึ่งพบว่าเมธานอล กลูโคส อะซิโตน และอะซิเทตตามลำดับช่วยเร่งให้เกิดการลดคลอรีนของ Aroclor 1242 ด้วยจุลชีพจากตะกอนของ Hudson River ที่มีประวัติการปนเปื้อนพีซีบีได้ เป็นที่น่าสังเกตุว่าอะซิเตทเป็นสารให้อิเลคตรอนที่ให้ผลดีต่อการลดคลอรีนของพีซีบีต่ำที่สุดในบรรดาสารอินทรีย์ที่ทดสอบทั้งหมดเหมือนเช่นกรณีที่พบในโครงการวิจัยนี้

4.3.2 ผลการศึกษาในตะกอนเหลว

คณะทำงานได้ศึกษาผลของการดูดซับของของแข็งที่มีต่อ Bioavailability ของพีซีบีต่อจุลชีพที่สามารถลดคลอรีนโดยใช้ตะกอนเหลว สารพีซีบีเป้าหมายคือ 2,3,4-ไตรคลอโรไบฟีนิลเนื่องจากพบว่าสามารถถูกย่อยสลายได้ในน้ำตะกอนภายในระยะเวลาที่ไม่นานนัก ผลที่ได้แสดงไว้ในตารางที่ 4.15 ซึ่งเมื่อเทียบกับกรณีของน้ำตะกอนในตารางที่ 4.11 แล้วจะพบได้ว่าการลดคลอรีนเกิดขึ้นในตะกอนเหลวได้ยากกว่าในน้ำตะกอนเหมือนเช่นในกรณีของเฮกซะคลอโรเบนซิน อันเป็นผลมาจากการดูดซับที่เพิ่มขึ้นจากปริมาณของแข็งที่สูงขึ้น อย่างไรก็ดีผลิตภัณฑ์ส่วนใหญ่ที่ได้ยังคงเป็น 2,4-ไดคลอโรไบฟีนิล

4.3.3 ผลการศึกษาในแบบจำลองลำน้ำ

คณะทำงานได้ทดสอบการย่อยสลายของ 2,3,4-ไตรคลอโรไบฟีนิลในแบบจำลองลำน้ำภายใต้สภาวะเช่นเดียวกับในกรณีของเฮกซะคลอโรเบนซิน เพื่อศึกษาความเป็นไปได้ในการลดคลอรีนในตะกอนตามธรรมชาติ แต่เลือกใช้ความเข้มข้นที่ 2 มิลลิกรัมต่อกิโลกรัมของแข็งแทนที่จะเป็น 5 มิลลิกรัมต่อกิโลกรัมของแข็งเหมือนในกรณีของเฮกซะคลอโรเบนซิน ทั้งนี้เพื่อลดความเป็นพิษของ 2,3,4-ไตรคลอโรไบฟีนิลที่อาจมีต่อจุลชีพในตะกอน ผลการศึกษาพบว่า

ตารางที่ 4.14 ผลของสารอาหารที่มีต่อการลดคลอรีนของ 2 มิลลิกรัมต่อลิตรของ 2,3,4-ไตรคลอโรไบฟีนิลในน้ำตะกอน

Site	Substrate	Lag Phase (weeks)	Complete Dechlorination (weeks)	Product
C1	Acetate+YE	6	>16	2,4-CBp
	Pyruvate+YE	6	>16	2,4-CBp
D1	Acetate+YE	10	>16	2,4-CBp
	Pyruvate+YE	6	>16	2,4-CBp
D4	Acetate+YE	10	>16	2,4-CBp
	Pyruvate+YE	6	>16	2,4-CBp
E1	No addition	10	>18	2,4-CBp
	Yeast Extract (YE)	16	>18	2,4-CBp
	Acetate	14	>18	2,4-CBp
	Lactate	4	>10	2,4-CBp
	Pyruvate	6	>18	2,4-CBp
	Acetate+YE	12	>14	2,4-CBp
	Lactate+YE	6	>10	2,4-CBp
	Pyruvate+YE	8	>14	2,4-CBp
E2	No addition	14	>18	2,4-CBp
	Yeast Extract (YE)	10	>18	2,4-CBp2
	Acetate	ND	ND	2,4-CBp
	Lactate	4	>10	2,4-CBp
	Pyruvate	14	>18	2,4-CBp
	Acetate+YE	5	>14	2,4-CBp
	Lactate+YE	4	>10	2,4-CBp
	Pyruvate+YE	6	>14	2,4-CBp
J1	Acetate+YE	4	>16	2,4-CBp
	Pyruvate+YE	6	>16	2,4-CBp

หมายเหตุ: -ND คือตรวจไม่พบ/ไม่เกิดการลดคลอรีน

ตารางที่ 4.15 การลดคลอรีนของ 2,3,4-ไตรคลอโรไบฟีนิลในตะกอนเหลว

Site	Lag Phase (weeks)	Complete Dechlorination (weeks)	Products
A1	5	14-22	2,4-CBp (major) & 2,3-CBp (minor)
A2	5	14-22	2,3-CBp
A3	5-8	14-22	2,4-CBp
A4	8	14-22	2,4-CBp
A5	5	14-22	2,4-CBp
A6	5-8	14-22	2,4-CBp
A7	5	14-22	2,4-CBp
A8	5	14-22	2,4-CBp
A9	5	14-22	2,4-CBp
A10	8	14-22	2,4-CBp
C1	9	21-24	2,4-CBp
D1	ND	ND	ND
D4	ND	ND	ND
E1	18-24	>24	2,4-CBp
E2	ND	ND	ND
F1	ND	ND	ND
J1	ND	ND	ND

หมายเหตุ: -ND คือตรวจไม่พบ/ไม่เกิดการลดคลอรีน

2,3,4-ไตรคลอโรไบฟีนิลที่สามารถถูกลดคลอรีนได้ในน้ำตะกอนและตะกอนเหลวไม่สามารถถูกย่อยสลายได้ในชั้นตะกอนตลอดระยะเวลาศึกษา 21 สัปดาห์ ชี้ให้เห็นว่าในสภาวะจริงตามธรรมชาติ 2,3,4-ไตรคลอโรไบฟีนิลมีความคงทนต่อการย่อยสลายทางชีวภาพแบบไร้อากาศมากกว่าเฮกซะคลอโรเบนซิน จึงเหมาะสมที่ถูกจัดให้เป็นสารมลพิษอินทรีย์คงทน ซึ่งต่างจากเฮกซะคลอโรเบนซินที่ไม่สมควรถูกจัดให้เป็นสารมลพิษอินทรีย์คงทนเพราะสามารถย่อยสลายได้ง่ายในสภาวะแวดล้อมของประเทศไทย

4.4 บทความวิชาการที่เผยแพร่

ผลงานวิจัยที่ได้รับจากโครงการนี้ได้มีการตีพิมพ์เผยแพร่ในวารสารวิชาการนานาชาติ 2 เรื่องและนำเสนอในที่ประชุมทางวิชาการในประเทศ 1 เรื่องดังต่อไปนี้ (manuscript ได้รวบรวมไว้ในภาคผนวก ข)

1) Chen I.M., Wanitchapichat W., Jirakittayakorn T., Sanohniti S., Sudjarid W., Wantawin C., Voranisarakul, and **Anotai J.**, Hexachlorobenzene Dechlorination by Indigenous Sediment Microorganisms, *Journal of Hazardous Materials* (in press).
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บทที่ 5 สรุปผลการศึกษา

ตลอดระยะเวลาหลายปีที่ผ่านมา คณะทำงานได้ศึกษาการลดคลอรีนของสารประกอบอินทรีย์คลอรีนเสี่ยงอันตรายสองกลุ่มคือ เฮกซะคลอโรเบนซินและโพลีคลอริเนเต็ดไบฟินิล โดยในกรณีของเฮกซะคลอโรเบนซินได้ข้อสรุปที่ชัดเจนแล้ว แต่ในกรณีของโพลีคลอริเนเต็ดไบฟินิลยังจำเป็นต้องมีการศึกษาเพิ่มเติมเนื่องจากโพลีคลอริเนเต็ดไบฟินิลย่อยสลายได้ยากและใช้เวลานานมาก ผลการศึกษาและผลงานที่สำคัญสามารถสรุปได้ดังต่อไปนี้

5.1 การย่อยสลายเฮกซะคลอโรเบนซิน

-เฮกซะคลอโรเบนซินสามารถย่อยสลายทางชีวภาพภายใต้สภาวะไร้อากาศได้เป็นอย่างดีทั้งในน้ำตะกอน ตะกอนเหลว และตะกอนโดยไม่จำเป็นต้องเติมสารอาหารหรือธาตุอาหารเสริม นอกจากนี้ยังตรวจพบการลดคลอรีนขึ้นในตะกอนก้นคลองหัวลำภูที่สภาวะในแหล่งกำเนิด (in situ) ตามธรรมชาติอีกด้วย เฮกซะคลอโรเบนซินที่ 5 มิลลิกรัมต่อกิโลกรัมแห้งของตะกอนสามารถถูกย่อยสลายจนหมดในตะกอนภายใต้สภาวะจำลองลำน้ำภายในเวลา 9 สัปดาห์ เป็นผลให้เฮกซะคลอโรเบนซินไม่เป็น “สารมลพิษอินทรีย์คงทน (Persistent Organic Pollutant)” ภายใต้สภาวะแวดล้อมของประเทศไทย

-การย่อยสลายเฮกซะคลอโรเบนซินเกิดขึ้นตามกลไกหลักเกือบทั้งหมดคือเป็นเพนตะคลอโรเบนซิน 1,2,3,5-เตตระคลอโรเบนซิน และ 1,3,5-ไตรคลอโรเบนซินตามลำดับ มีเพียงส่วนน้อยเท่านั้นที่ย่อยสลายผ่านกลไกรองคือเป็นเพนตะคลอโรเบนซิน 1,2,4,5-เตตระคลอโรเบนซิน 1,2,4-ไตรคลอโรเบนซิน และ 1,4-ไดคลอโรเบนซินตามลำดับ

-อุณหภูมิเป็นปัจจัยสำคัญที่สุดที่ทำให้จุลชีพในตะกอนจากลำน้ำในประเทศไทยซึ่งมีสภาพภูมิอากาศแบบ Tropical Climate มีความสามารถในการลดคลอรีนของเฮกซะคลอโรเบนซินสูงกว่าจุลชีพในงานวิจัยอื่นๆที่ดำเนินการในประเทศที่พัฒนาแล้วซึ่งมีภูมิอากาศแบบ Temperate Climate หรือ Continental Climate ผลการศึกษาพบว่าอุณหภูมิที่เหมาะสมสำหรับการย่อยสลายเฮกซะคลอโรเบนซินอยู่ในช่วง 30 ถึง 40 $^{\circ}$C ซึ่งใกล้เคียงกับอุณหภูมิห้องของประเทศไทย เป็นผลให้จุลชีพกลุ่มที่ย่อยสลายเฮกซะคลอโรเบนซินได้สามารถเจริญเติบโตได้ดีตามธรรมชาติจนมีจำนวนประชากรมากพออยู่ในตะกอนตามลำน้ำทั่วไป ในขณะที่ประเทศที่พัฒนาแล้วจะมีอุณหภูมิในช่วงฤดูหนาวต่ำมากและเป็นเวลายาวนาน ทำให้จุลชีพกลุ่มดังกล่าวไม่สามารถเจริญเติบโตหรือรักษาความสามารถในการลดคลอรีนไว้ได้

-จุลชีพที่ลดคลอรีนของเฮกซะคลอโรเบนซินได้เป็นจุลชีพที่สามารถพบได้ทั่วไปในตะกอนจากแหล่งน้ำในประเทศไทย ผลการศึกษาชี้ว่าจุลชีพกลุ่มดังกล่าวน่าจะเป็นแบคทีเรียกลุ่มเมธาโนเจนและแบคทีเรียแกรมบวกที่มีความสามารถในการทนต่อการยับยั้งของ vancomycin ได้ดี

-การลดคลอรีนของเฮกซะคลอโรเบนซินทางชีวภาพแบบไร้อากาศเป็นกลไกของเมตาบอลิซึมร่วม (co-metabolism) ซึ่งจลนพลศาสตร์ของการลดเฮกซะคลอโรเบนซินสามารถอธิบายได้ด้วยสมการของ Michaelis-Menton Kinetics โดยมีค่าคงที่อัตราการลดคลอรีนสูงสุดปรากฏ (apparent maximum dechlorination rate constant) และค่าคงที่ครึ่งอิ่มตัว (half-saturation constant) ของเฮกซะคลอโรเบนซินเท่ากับ 0.45-0.73 มิลลิกรัมต่อลิตรต่อวันและ 3.2-17.2 มิลลิกรัมต่อลิตรตามลำดับ

5.2 การย่อยสลายโพลีคลอริเนเต็ดไบฟีนิล

-จากโพลีคลอริเนเต็ดไบฟีนิล 21 คอนเจนเนอร์ที่ทดสอบมีเพียง 2,3,4-ไตรคลอโรไบฟีนิลคอนเจนเนอร์เดียวที่สามารถถูกย่อยสลายทางชีวภาพแบบไร้อากาศได้ดีในทุกกรณีที่ทดสอบแต่เกิดขึ้นช้ามาก โดยที่ความเข้มข้น 2 มิลลิกรัมต่อลิตรในน้ำตะกอนต้องใช้เวลาในการย่อยสลายทั้งหมดไม่น้อยกว่า 10 สัปดาห์ขึ้นไป ผลิตภัณฑ์ที่ได้คือ 2,4-ไดคลอโรไบฟีนิล ส่วน 3,4,5-ไตรคลอโรเบนซินย่อยสลายได้เป็นส่วนน้อยเพียงบางชุดทดสอบ โดยผลิตภัณฑ์ที่ได้จากการลดคลอรีนคือ 3,5-ไดคลอโรไบฟีนิล ส่วนคอนเจนเนอร์ที่เหลือมีเพียง 2,4,4'-ไตรคลอโรไบฟีนิล, 2,4,2',5'-, 2,3,2',5'- และ 2,3,4,4'-เตตระคลอโรไบฟีนิลเท่านั้นที่สามารถย่อยสลายได้เป็น 2,4-ไดคลอโรไบฟีนิล, 2,4,2'-, 2,3,2'- และ 2,4,4'-ไตรคลอโรไบฟีนิลตามลำดับ

-ในกรณีที่มีคลอรีน 3 อะตอมเรียงกันในวงไบฟีนิลจะพบว่ากลไกในการลดคลอรีนเกิดขึ้นที่คลอรีนอะตอมที่อยู่กลาง ส่วนในกรณีอื่นๆพบว่าคลอรีนอะตอมในตำแหน่งเมตาจะถูกดึงออกได้ดีกว่าตำแหน่งพารา และไม่พบการลดคลอรีนในตำแหน่งออโธเลย

-การจัดให้โพลีคลอริเนเต็ดไบฟีนิลเป็นสารมลพิษอินทรีย์คงทนในประเทศไทยมีความเหมาะสมแล้ว ถึงแม้ว่าผลการทดสอบพบว่าบางคอนเจนเนอร์จะสามารถถูกย่อยสลายได้บ้าง แต่คอนเจนเนอร์ส่วนใหญ่ย่อยสลายทางชีวภาพภายใต้สภาวะไร้อากาศได้ช้ามากหรือไม่ได้เลย ดังนั้นเมื่อรูปแบบของสารประกอบโพลีคลอริเนเต็ดไบฟีนิลที่ผลิตทางการค้าเป็นของผสมของหลายๆคอนเจนเนอร์เข้าด้วยกัน จึงมีโอกาสตกค้างอยู่ในธรรมชาติได้เป็นเวลานานจนอาจก่อให้เกิดผลกระทบด้านลบต่อสิ่งแวดล้อมและสิ่งมีชีวิตได้

5.3 ผลลัพธ์จากโครงการวิจัย

-ผลงานตีพิมพ์ในวารสารวิชาการนานาชาติ 2 เรื่องที่เกี่ยวข้องกับการลดคลอรีนของเฮกซะคลอโรเบนซิน
-ผลงานนำเสนอในที่ประชุมวิชาการระดับชาติ 1 เรื่องที่เกี่ยวข้องกับการลดคลอรีนของโพลีคลอริเนเต็ดไบฟินิล

5.4 งานวิจัยในอนาคต

เนื่องจากการย่อยสลายทางชีวภาพแบบไร้อากาศของโพลีคลอริเนเต็ดไบฟินิลเกิดขึ้นช้ามาก คณะวิจัยยังคงตามผลการลดคลอรีนที่เกิดขึ้นจากการทดสอบภายใต้สภาวะต่างๆ ยิ่งไปกว่านั้นก่อนสิ้นสุดโครงการได้มีการศึกษาถึงความเป็นไปได้ที่สารอินทรีย์ที่มีโครงสร้างคล้ายคลึงกับโพลีคลอริเนเต็ดไบฟินิลจะสามารถกระตุ้นให้จุลชีพสร้างเอนไซม์ที่เกี่ยวข้องในการย่อยสลายโพลีคลอริเนเต็ดไบฟินิลขึ้น ผลทดสอบในเบื้องต้นพบว่าเป็นที่น่าพอใจและมีความเป็นไปได้สูง นอกจากนี้คณะทำงานยังได้ทำการเพิ่มจำนวนจุลชีพที่สามารถย่อยสลายเฮกซะคลอโรเบนซินและโพลีคลอริเนเต็ดไบฟินิลได้เพื่อนำไปวิเคราะห์หาเผ่าพันธุ์ (species) จุลชีพที่เกี่ยวข้องในรายละเอียดโดยใช้เทคนิคทางจุลชีววิทยาที่เรียกว่า “Denaturing Gradient Gel Electrophoresis (DGGE)” โดยมีนักศึกษาในระดับปริญญาโทและเอกทำการศึกษาต่อไป

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ภาคผนวก

ภาคผนวก ก
ภาพสถานที่และการเก็บตัวอย่างตะกอนและน้ำ

ก.1 คลองหัวลำภูซึ่งรับน้ำทิ้งจากนิคมอุตสาหกรรมบางปู ถนนสุขุมวิท สมุทรปราการ


รูปที่ ก.1.1 บริเวณจุดเก็บตัวอย่าง Site A1 ริมถนนสุขุมวิทจะเห็นได้ถึงความแตกต่างของน้ำในคลองซึ่งขึ้นอยู่กับการระบายน้ำทิ้งของนิคมอุตสาหกรรมบางปู


รูปที่ ก.1.2 บริเวณจุดเก็บตัวอย่าง Site A2 จะเห็นได้ถึงความแตกต่างของน้ำในคลองซึ่งขึ้นอยู่กับการระบายน้ำทิ้งของนิคมอุตสาหกรรมบางปู

รูปที่ ก.1.3 บริเวณจุดเก็บตัวอย่างและการเก็บตัวอย่างน้ำและตะกอนของ Site A3

รูปที่ ก.1.4 บริเวณจุดเก็บตัวอย่างและการเก็บตัวอย่างน้ำและตะกอนของ Site A4 รวมถึงการปาดชั้นหน้าตะกอนทิ้งก่อนเก็บตัวอย่าง

รูปที่ ก.1.5 การเก็บตัวอย่างน้ำและตะกอนของ Site A5

รูปที่ ก.1.6 การเก็บตัวอย่างน้ำและตะกอนของ Site A6

รูปที่ ก.1.7 การเก็บตัวอย่างน้ำและตะกอนของ Site A7

รูปที่ ก.1.8 การเก็บตัวอย่างน้ำและตะกอนของ Site A8

รูปที่ ก.1.9 การเก็บตัวอย่างตะกอนและปลาตีนที่พบที่ Site A9

รูปที่ ก.1.10 สภาพพื้นที่ของจุดเก็บตัวอย่าง Site A10 ในช่วงน้ำลงและน้ำขึ้น

ก.2 คูระบายน้ำข้างถนนบางเมฆขาว สมุทรปราการ

รูปที่ ก.2.1 สภาพโดยทั่วไปของคูน้ำ

รูปที่ ก.2.2 บริเวณจุดเก็บตัวอย่าง Site B1

รูปที่ ก.2.3 บริเวณจุดเก็บตัวอย่าง Site B2

รูปที่ ก.2.4 บริเวณจุดเก็บตัวอย่าง Site B3

รูปที่ ก.2.5 บริเวณจุดเก็บตัวอย่าง Site B4

ก.3 คลองใกล้โรงไฟฟ้าพระนครใต้

รูปที่ ก.3.1 บริเวณที่เก็บตัวอย่างในคลองใกล้โรงไฟฟ้าพระนครใต้ (Site C1) ในช่วงที่มีการสร้างสะพาน

รูปที่ ก.3.2 บริเวณที่เก็บตัวอย่างในคลองใกล้โรงไฟฟ้าพระนครใต้ (Site C2) ในช่วงที่สร้างสะพานแล้วเสร็จ

ก.4 คลองรับน้ำทิ้งจากนิคมอุตสาหกรรมบางพลี

รูปที่ ก.4.1 จุดระบายน้ำทิ้งของนิคมอุตสาหกรรมบางพลีลงสู่คลอง

รูปที่ ก.4.2 สภาพทั่วไปของคลองและจุดเก็บตัวอย่าง Site D1

รูปที่ ก.4.3 สภาพทั่วไปของคลองบริเวณจุดเก็บตัวอย่าง Site D2

รูปที่ ก.4.4 บริเวณจุดเก็บตัวอย่าง Site D3

รูปที่ ก.4.5 สภาพโดยทั่วไปของคลองบริเวณจุดเก็บตัวอย่าง Site D4

รูปที่ ก.4.6 ลักษณะตะกอนที่เก็บตัวอย่าง

ก.5 คลองข้างถนนตำหรุ-บางพลี สมุทรปราการ

รูปที่ ก.5.1 สภาพโดยทั่วไปของคูน้ำในบริเวณจุดเก็บตัวอย่าง Site E1

รูปที่ ก.5.2 สภาพโดยทั่วไปของคลองข้างถนนตำหรุ-บางพลีบริเวณจุดเก็บตัวอย่าง Site E2

ก.6 คลองบางฝ้ายซึ่งรับน้ำทิ้งจากนิคมอุตสาหกรรมลาดกระบัง

รูปที่ ก.6.1 สภาพโดยทั่วไปของคลองบางฝ้ายบริเวณจุดเก็บตัวอย่าง Site F1

รูปที่ ก.6.2 การวัดออกซิเจนละลายในน้ำ

ก.7 คลองแพรกษา สมุทรปราการ

รูปที่ ก.7.1 สภาพโดยทั่วไปของคลองแพรกษาบริเวณจุดเก็บตัวอย่าง Site G1

ก.8 คูน้ำข้างบริษัท ไทยเซ็นทรัลเคมีคัล จำกัด (มหาชน) ถนนสุขสวัสดิ์ สมุทรปราการ

รูปที่ ก.8.1 สภาพของคูน้ำ

รูปที่ ก.8.2 บริเวณจุดเก็บตัวอย่าง Site H1

รูปที่ ก.8.3 บริเวณจุดเก็บตัวอย่าง Site H2

รูปที่ ก.8.4 ลักษณะตะกอนตัวอย่าง

ก.9 คูน้ำข้างถนนสุขสวัสดิ์ สมุทรปราการ

รูปที่ ก.9.1 สภาพของคูน้ำและบริเวณที่เก็บตัวอย่าง Site I1

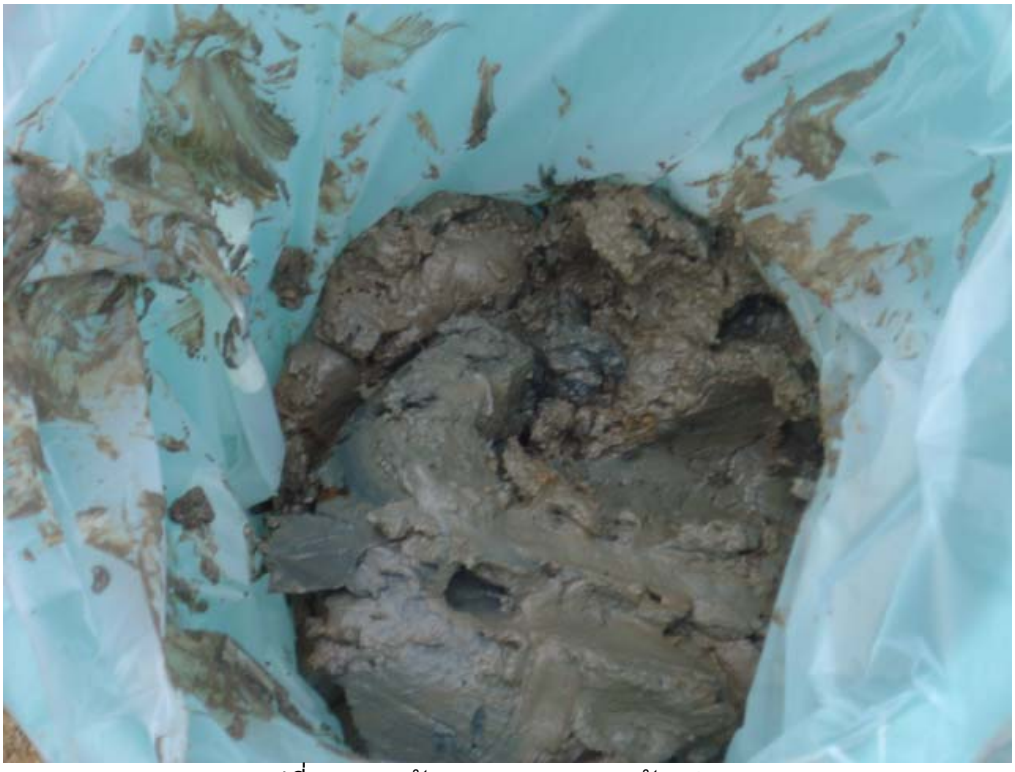
ก.10 คลองบางปลากด ถนนสุขสวัสดิ์ สมุทรปราการ

รูปที่ ก.10.1 สภาพทั่วไปของคลองบางปลากด

รูปที่ ก.10.2 สภาพพื้นที่บริเวณจุดเก็บตัวอย่าง Site J1

รูปที่ ก.10.3 สภาพพื้นที่บริเวณจุดเก็บตัวอย่าง Site J2

รูปที่ ก.10.4 ฟองก๊าซจากการย่อยสลายภายใต้สภาวะไร้อากาศที่ผุดขึ้นในระหว่างเก็บตะกอน



รูปที่ ก.10.5 ลักษณะของตะกอนตัวอย่าง

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Hexachlorobenzene dechlorination by indigenous sediment microorganisms

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ABSTRACT

Indigenous microbes from the sediments, whether contaminated with hexachlorobenzene (HCB) or not, could dechlorinate HCB effectively without any acclimation and supplemental nourishment. Temperature seriously affected the HCB-dechlorination: within the measured 15–45 °C span, the optimum range was between 30 and 35 °C. Sulfate-reducing bacteria (SRB), denitrifiers, and acetogens might not be directly involved in the HCB dechlorination. However, the SRB retarded subsequent dechlorination of pentachlorobenzene to tetra- and trichlorobenzenes. Some vancomycin-resistant gram-positive bacteria and methanogens were most likely to be the HCB-dechlorinators. The dechlorination followed the Michaelis–Menten behavior with the k'_m and K_{HCB} between 0.45–0.73 mg L^{-1} day^{-1} and 3.2–17.2 mg L^{-1}, respectively. These findings suggest a potential HCB treatment and cleanup for wastewater and contaminated site.

1. Introduction

Hexachlorobenzene (HCB) was produced and used globally as a fungicide and an industrial synthetic material before production was banned several decades ago. Because of its bioaccumulation and persistence properties, as well as its toxicity, HCB was classified as one of the 12 persistent organic pollutants (POPs) by the United Nations Stockholm Convention. HCB has been found to contaminate in many places worldwide including Thailand as a result of uncontrolled release. HCB was sporadically detected in the sediment of the Hua Lum Poo Canal in Samuth Prakarn Province, Thailand, which receives treated effluent from nearby industrial estate and factories, even though the direct use of HCB in Thailand has been prohibited since 1980 [1,2]. This finding is comprehensible since HCB is also unintentionally generated as a byproduct from the manufacture of the chlorinated pesticides such as atrazine and simazin [3,4]. Of more concern is that this canal eventually empties into the Gulf of Thailand, posing a threat of HCB contamination and bioaccumulation in the coastal bivalves that are consumed by local people.

Environmental degradation of HCB under anaerobic conditions in soil and sediment is possible but considerably slow. Beurskens et al. [5] found an 80% loss of HCB with an increase in the by-products of 1,3,5-trichlorobenzene (1,3,5-TCB) and 1,3-dichlorobenzene (1,3-DCB) during a 20-year period of in situ degradation in Lake Ketelmeer, a sedimentation area of the Rhine River. The half lives of HCB in soil and groundwater were reported to be 3–6 and 5.3–11.4 years, respectively [6]. Prytula and Pavlostathis [7] evaluated the HCB-dechlorination ability of the sediment slurry collected from an HCB-contaminated tributary without any acclimation or enrichment and found that only 43% of HCB was dechlorinated in 481 days at 23 °C in the dark. Rosenbrock et al. [8] could obtain only 40% chloride release in the rich-organic soil slurry spiked with HCB in 140 days, whereas no dechlorination activity was observed in the low-organic soil slurry. Chen et al. [9] worked with four non-HCB contaminated sediment slurries and found only two slurries could initiate HCB-dechlorination with a lag phase of 90 days. These data, however, show different results from our previous study in which 17 mg kg^{-1} dry solids of HCB could be rapidly and completely degraded within 60 days by the indigenous microbial consortium in the sediment slurries collected along the Hua Lum Poo Canal and its mouth without any supplementation of organics or nutrients [1]. The degradation followed the major pathway proposed by Fathepure et al. [10], i.e., HCB → pentachlorobenzene (QCB) → 1,2,3,5-tetrachlorobenzene (TeCB) → 1,3,5-TCB. This finding corresponded very well with the field data in which 1,3,5-TCB was found together with HCB in the upstream sediments of this canal [1].

The objective of this work was to investigate into more details on the factor causing the microbes in the sediment of the Hua Lum Poo Canal to be more powerful in HCB-dechlorination than others. Microbial groups involving in the HCB-dechlorination and

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the dechlorination kinetics were also determined. The information reported here provides a better understanding regarding the reductive dechlorination of HCB, which can lead to a promising treatment or cleanup technique in the future.

2. Materials and methods

2.1. Chemicals

Chlorinated benzene congeners (CBs) including monochlorobenzene (MCB), 1,2-, 1,3-, and 1,4-DCBs, 1,2,3-, 1,2,4-, and 1,3,5-TCBs, 1,2,3,4-, 1,2,3,5-, and 1,2,4,5-TeCBs, QCB, and HCB were purchased from Seelze, Germany. The microbial inhibitors including bromoethanesulfonic acid (BES) and vancomycin (VAN) were obtained from Sigma Chemical Co., USA. The 99.5% acetone and n-hexane (Labscan Asia, Co., Ltd., Thailand) were used for preparing an HCB solution and CBs standards/extraction liquid, respectively. All other chemicals were analytical grade and supplied by Merck KGaA, Germany.

2.2. Sediment and canal water sampling

Sediment and canal water samples from two sites (H1 and H2) along the Hua-Lam-Poo Canal that possessed the highest HCB-dechlorination activity according to Anotai et al. [1] were used in this study. The top few centimeters of the sediment surface were carefully removed, and the lower layer was scraped and packed in a plastic bag. Canal waters were sampled and stored in the containers. Both sediment and water samples were stored in the 4 °C cold storage until use.

2.3. Microbial preparation

Sediment slurry of each site was prepared by thoroughly mixing the sediment and canal water at the ratio of 1:1 (v/v) by hand for 2 min and was allowed to settle for 30 min. The upper supernatant was withdrawn by a 100-mL glass syringe with a 22G × 2 hypodermic needle (0.7 mm opening), injected into a 1000-mL serum bottle, and purged with nitrogen gas before use. For dechlorination experiments, 50 mL of sediment slurry were transferred to several 100-mL serum bottles in a nitrogen glove box to prevent oxygen interference and sealed with butyl rubber stoppers and alumina-caps. One bottle from each experiment set was sterilized three times in an autoclave and used as the control.

2.4. Dechlorination experiments

To initiate the experiment, an appropriate amount of stock HCB solution, along with a specific amount of the individual chemical to be studied, was injected into the serum bottles. All serum bottles were kept in the dark at room temperature (28–31 °C with an average of 30 °C). To study temperature effects, refrigerators (at 15 and 20 °C), air-incubators (at 35 and 40 °C), and water baths at 45 °C were used to control the temperature. All bottles were shaken by hand on a daily basis. The experiments were conducted in duplicate with a sterilized control.

2.5. Sampling and analysis

To determine HCB and its dechlorination by-products, HCB from the sediment slurry was extracted according to the method described by Chen et al. [11] which provided the recovery between 89 and 98% for all 12 chlorobenzenes. An extraction test has been performed with 1-h HCB spiked sediment slurry in order to confirm the reliability of this method. The HCB recovery was between 88 and 95%. In addition, another test was also conducted using non-biodegradable and highly hydrophobic 2,3,4,2′,4′,5′- and 2,3,6,2′,4′,5′-chlorobiphenyls to verify the consistency of this extraction method over an adsorption period of 18 weeks. The recover was between 88 and 112% for 2,3,4,2′,4′,5′-chlorobiphenyl and 91 and 110% for 2,3,6,2′,4′,5′-chlorobiphenyl with no less-chlorinated intermediate detected. Hence, this extraction method is proven to be reliable. At a predetermined time, 2 mL of the sediment slurry was taken by a glass syringe with a 22G × 2 hypodermic needle (using the same needle size used to withdraw the sediment slurry from the sediment-water mixture in order to ensure a homogeneous sample) and injected into an extraction tube containing 0.2 mL of 6N NaOH and 2 mL of n-hexane. The tube was then shaken by hand 100 times, followed by 10 min of sonication, and then centrifuged at 4000 rpm for 5 min. The upper-layer of n-hexane was withdrawn as much as possible into a 5-mL analyzing tube. The remaining mixture in the extraction tube was then re-extracted twice more following the same procedure. At the third extraction, the upper-layer of n-hexane was pulled out and filled the analyzing tube up to the 5-mL mark. A small amount of anhydrous Na_2SO_4 was added to remove moisture before being analyzed by gas chromatography. The 6890N Network GC system (Agilent Technologies, USA) was equipped with an electron capture detector (ECD) and a capillary column DB-5 fused silica with 0.25 mm diameter and 30 m length (Agilent Technologies, USA). The oven temperature was initially maintained at 80 °C for 5 min, raised to 140 °C at the rate of 3 °C min^{-1} and sequentially to 240 °C at the rate of 10 °C min^{-1}, and hold for 8 min. The temperature for the injector and detector were set at 240 and 280 °C, respectively. The carrier and make up gases were helium and nitrogen at the average linear flow rates of 20 and 60 mL min^{-1}, respectively. All qualifications and quantifications were performed with an external standard. Methane in the headspace gas was determined by the Gas Chromatograph GC-8A (Shimadzu Corporation, Japan). Sediment slurry characteristics were analyzed according to Standard Methods [12].

3. Results and discussion

3.1. Background contamination and sediment slurry characteristics

Trace amounts of HCB were found in the sediments of both sites at 0.26 and 0.15 mg kg^{-1} dry solids for H1 and H2, respectively. In addition, 0.16 mg kg^{-1} dry solids of 1,3,5-TCB, which has never been produced or used in a commercial scale, was also found in the sediment from H1. This implies that the microbes at both sites should be acclimated to HCB to some degree and be able to dechlorinate HCB in situ. The characteristics of raw sediment slurries were shown in Table 1 illustrating that most organics were in the solid phase. Volatile organics, part of which representing the microbial mass, were only accounted for at 9% and 13% at H1 and H2, respectively. Nitrogen and phosphorus in the sediment slurries were sufficient for anaerobic digestion according to the acceptable COD:N:P ratio of 250:5:1–700:5:1 [13]. Chloride was quite high due to the intrusion of seawater at the sampling time. The pH of the mixtures was in the neutral range appropriate for microbial activities. Pre-tests with nutrient and organic supplements of either yeast extract, glucose, pyruvate, lactate, acetate, formate, or essential minerals revealed no significant improvement in the dechlorination process. Hence, the existing composition of sediment slurries was already suitable for the reductive dechlorination of HCB.

3.2. Reductive dechlorination of HCB

Sediment slurries from both H1 and H2 could dechlorinate 2 mg L^{-1} HCB completely in 70 days at the average room temper-

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Table 1
Characteristics of raw sediment slurries.

Site	pH	$Cl^-_{soluble}$ (mg L^{-1})	SS (mg L^{-1})	VSS (mg L^{-1})	COD (mg L^{-1})		Nitrogen (mg L^{-1})		Phosphorus (mg L^{-1})	
					Soluble	Total	Soluble	Total	Soluble	Total
H1	7.0	8938	228,300	19,600	584	42,985	13	896	2	66
H2	7.2	4080	158,500	21,200	215	50,149	12	1,036	1	77

ature of 30 °C as shown in Fig. 1. In contrast, no dechlorination occurred in the sterilized control bottles, i.e., steady HCB concentration and no intermediates detected, implying that the reduction of HCB in the experimental sets should derive from microbial activities. This performance was more impressive than other studies working with the sediment slurries from both HCB-contaminated and non-contaminated sites [7–9]. The degradation pathway followed the major pathway from HCB to QCB, 1,2,3,5-TeCB and 1,3,5-TCB as suggested by Fathepure et al. [10]. No 1,2,4-TCB and DCBs which are the products from the minor dechlorination pathway were detected. Certain loss of chlorobenzenes from the aqueous phase was also detected which mainly due to the transfer of volatile 1,3,5-TCB to the headspace of serum bottle similar to the observation of other studies [6,9,11,14]. The dechlorination performance obtained in this study was consistent with our preliminary work [1] implying that the microbes in the sediments of this canal could efficiently maintain their dechlorination ability.

3.3. Effect of temperature on HCB-dechlorination

It is interesting to determine the key factor enhancing the HCB-dechlorination ability of the microbes in this canal. One possible factor causing the differences in HCB-dechlorination between this study and others was the temperature. Most of Thailand has a tropical climate in which the coldest month temperature is higher than 18 °C. According to Thai Meteorological Department, the 30-year monthly averages for night-time and day-time in Samuth Prakarn Province are 26.3 and 30.3 °C, respectively (day-time temperatures during the experimental period of this study were between 28 and 31 °C with an average of 30 °C). On the other hand, most developed countries in which the HCB-dechlorination has been studied are in the temperate-climate (the coldest month average between −3 and 18 °C) or continental-climate (the coldest month average below −3 °C) zones according to the Köppen–Geiger climate classification system. As a result, the native microbial consortium in the sediment of the Hua Lum Poo Canal as well as other streams in Thailand should be diverse from those of developed countries and might possibly lead to an explanation for the differences in HCB-dechlorination. To test whether the temperature was one of the major factors controlling the HCB-dechlorination activity, the sediment slurries without any supplements were spiked with 2 mg L^{-1} of HCB and incubated at various temperatures from 15 to 45 °C. The outcomes showed that the optimum temperatures were between 30 and 40 °C for H1 and 30 and 35 °C for H2 (Table 2). The dechlorination performance drastically deteriorated as the temperature became lower or higher. The lag phase and complete dechlorination period extended approximately two times or more as the temperatures rose above or dropped below the optimum range. This finding serves as solid evidence that temperature plays a major role in classifying and characterizing microbial consortium and activity regarding on HCB-dechlorination in natural stream sediment. Further investigation was conducted to verify the effect of temperature by using the sediments collected from four other streams which were not contaminated with HCB. The results shown in Fig. 2 indicate that the indigenous microbial consortiums in these stream

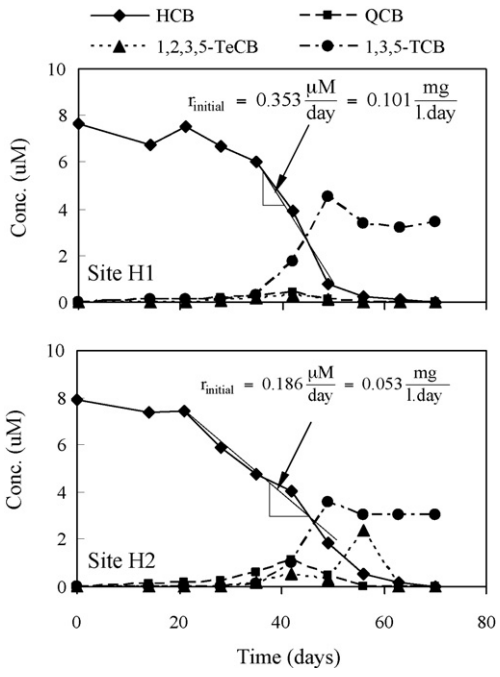

Fig. 1. Profiles of HCB and its intermediates with an initial HCB concentration of 2 mg L^{-1}.

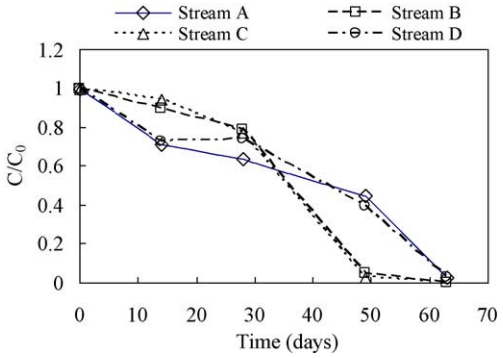

Fig. 2. HCB-dechlorination in non-contaminated sediment.

Table 2
HCB-dechlorination under various temperatures.

Site	Temperature (°C)	Lag phase (days)	Complete dechlorination (days)
H1	15	35 (42)	>154 (>154)
	20	28 (35)	126 (126)
	30	14 (7)	70 (70)
	35	7 (7)	63 (63)
	40	7 (7)	63 (70)
	45	14 (35)	>154 (>154)
H2	15	70 (35)	>154 (>154)
	20	49 (49)	161 (161)
	30	14 (14)	70 (70)
	35	7 (14)	70 (70)
	40	14 (14)	>154 (>154)
	45	42 (35)	>154 (>154)

Note: Numbers in the parenthesis are duplication.

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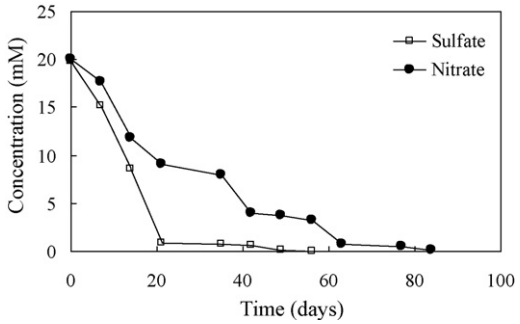

Fig. 3. Consumption profile of added electron acceptors.

sediments could readily dechlorinate 2 mg L^{-1} HCB with no acclimation or supplemental nourishment at room temperature similar to those from H1 and H2. These results suggest that temperature plays an important role on the dechlorination of HCB which is similar to the study of Chang et al. [6] who found the optimum temperatures for HCB-dechlorination to be between 29 and 37 °C and the dechlorination ability ceased at 18 and 45 °C.

From these results, it implies that HCB in the tropical-climate environment with moderate ambient temperatures might not be as persistent as in the temperate-climate and continental-climate regions due to the differences in native microbial matrix and activities. Biologically anaerobic processes, which typically have a long sludge detention time, such as an anaerobic filter or upflow anaerobic sludge blanket followed by an aerobic process might be a feasible alternative to purify HCB-contaminated wastewater. Remediation of HCB-contaminated sites is also promisingly possible if suitable conditions are provided. The identified end product was 1,3,5-TCB for all studied temperatures, neither 1,2,4-TCB nor DCBs was detected. Hence, the dechlorination mechanism still followed the major pathway. Nevertheless, substantial dissimilarity in the complete dechlorination period at 40 °C between the sediments from H1 and H2 suggested that the microbial consortium existing in these two sites were different, which was in agreement with the results from the kinetic studies in later section.

3.4. Characterization of dechlorinating microorganisms

This section attempted to determine the principal microbes responsible for HCB-dechlorination. Only the sediment slurry from H2 was used for characterization purpose. The first experimental scenario in this section aimed to determine the involvement of sulfate-reducing bacteria (SRB) and denitrifiers (DN) in the dechlorination of HCB to its less-chlorinated products. Sediment slurry with 2 mg L^{-1} of HCB was added with 20 mM of either $NaNO_3$ or Na_2SO_4 to stimulate the activities of DN or SRB, respectively. Nitrate reduction during the incubation period was slower than sulfate, as shown in Fig. 3, which implied that the SRB in this sediment were more active than the DN. This is plausible since the collected bottom sediment was under anaerobic conditions for a very long period of time. An anoxic state, which uses nitrite or nitrate as an electron acceptor, is unlikely to occur intensively in this rich organic sediment deposited a few centimeters below the sediment–water interface. No significant difference in HCB-dechlorination between these two supplement and control sets was observed, i.e., QCB appeared at the same time on day 14 (Table 3) with comparable apparent concentration between 0.018 and 0.019 mg L^{-1}. During this initial period, the SRB and DN were very active, interpreting from the zero-order disappearance rates of sulfate and nitrate (Fig. 3). This result suggested that neither SRB nor DN directly engaged in or serious interfered with the HCB-dechlorination since the addition of sulfate or nitrate to promote their activities neither

Table 3
Effects of electron donors on HCB-dechlorination of the sediment slurry from Site H2.

Electron acceptor	Appearance time (days)		
	QCB	1,2,3,5-TeCB	1,3,5-TCB
No addition	14	42	56
20 mM of $NaNO_3$	14	56	56
20 mM of Na_2SO_4	14	70	98

enhanced nor retarded the transformation of HCB to QCB. Working with HCB-adapting sediment, Chen et al. [14] also found that 30 mM sulfate did not interfere with HCB-dechlorination; however, they suggested that under limiting electron donors SRB might compete with HCB-dechlorinators for substrate, thus hampering the HCB-dechlorination. The impact of nitrate seemed to be unclear on QCB transformation to 1,2,3,5-TeCB and 1,3,5-TCB. The appearance time for 1,2,3,5-TeCB in the presence of nitrate was 14 days longer than the control without nitrate, but was the same for 1,3,5-TCB. It is important to note that the sample on day 56 was the first sampling after day 42; hence, they could not be definitely differentiated. The presence of sulfate, however, significantly prolonged the transformation of QCB to 1,2,3,5-TeCB and subsequently to 1,3,5-TCB. The occurrence times for 1,2,3,5-TeCB and 1,3,5-TCB shifted from 42 to 70 days and from 56 to 98 days, respectively. This implies that the activity of SRB noticeably interfered with QCB- and 1,2,3,5-TeCB dechlorinators. As a result, it indicates that the population responsible for chlorobenzene dechlorination differed at least in some species from the QCB- and 1,2,3,5-dechlorinators. This result is in agreement with the study of Chen et al. [11].

To evaluate the role of methanogens on HCB-dechlorination, the selective methanogenic inhibitor, bromoethanesulfonic acid (BES) [6], was inoculated into the serum bottle at various concentrations. It was found that only 5 mM of BES could notably suppress the methanogenic activity. Accumulated methane in the headspace of the serum bottle on day 78 in the control set without BES was 38% which was much higher than those in the BES amended set of less than 1%. Nevertheless, more than 90% of the HCB was still dechlorinated, though at a lower rate, as shown in Fig. 4. This suggests two possibilities: first, the dechlorinating step was directly executed by methanogenic bacteria only, but maybe a variety of different species of methanogens can play this role. Therefore, whenever one or some methanogenic bacteria regained their activity and began to produce methane even slightly, these methanogens could be able to trigger the dechlorination of HCB. Second, while the methanogens played a major role in HCB-dechlorination (reduction rate was steeper in the control set), other microorganisms in the sediment slurry might also involved. As the BES was increased to 10 and 50 mM, HCB was still dechlorinated. Only when 250 mM was applied did the dechlorination stop completely. Middledorp

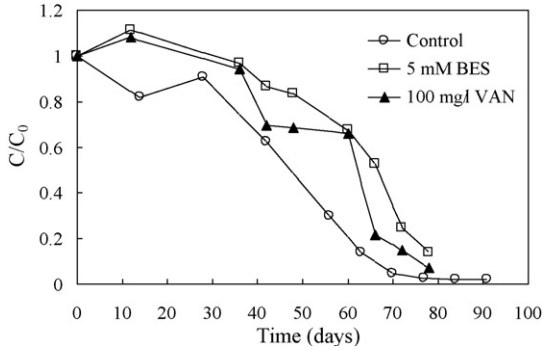

Fig. 4. Dechlorination profile for HCB with and without inhibitors.

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et al. [15] found that BES at high concentration also affected the microbial species other than methanogens, possibly suppressing the non-methanogenic HCB-dechlorinators in the sediment slurry. The results from this study filled the gap between two totally different observations from other research groups. Pavlostathis and Prytula [16] reported that methane production was inhibited in a BES-amended culture, but sequential reductive dechlorination of HCB still occurred at a comparable rate as in the non-amended culture, indicating that methanogens were not involved in the dechlorination process. On the other hand, the data from the BES-amended studies of Chang et al. [6] and Chen et al. [14] strongly suggested that methanogens were the sole HCB-dechlorinators in their acclimated cultures. Combining these facts together with the results from this study, it can be concluded that HCB-dechlorinators should consist of methanogens as well as other microbial groups. In the studies of Chang et al. [6] and Chen et al. [14], methanogens served as the sole HCB-dechlorinators in their cultures. As a result, once the methanogens were inactivated by BES, the dechlorination of HCB was also terminated. In the study of Pavlostathis and Prytula [16], however, the dechlorination occurred via the activities of the microbial species other than methanogens. Hence, the inhibition of methanogenic activities did not completely block HCB-dechlorination. In this study, the sediment slurry contained both methanogens and other HCB dechlorinating species; thus, even though the methanogens were almost completely inactivated by the BES, the remaining dechlorinators that were not susceptible to BES still transformed HCB to QCB; however, at the lower rate as compared to when both microbial groups were active.

Further investigation on HCB-dechlorinators was conducted by using vancomycin (VAN), a strong bactericide on gram-positive bacteria including most acetogens. The results revealed that 100 mg L^{-1} of VAN moderately retarded the dechlorination performance shown in Fig. 4. Partial pressure of methane in the headspace also reduced from 38% to 16% in the presence of VAN during the same incubation period. This implies that acetogenic activities that transformed volatile fatty acids to acetic acid for methanogenic uptake were partially affected and consequently retarded methane formation. Once the methanogenic activities subsided, the dechlorination process was also decelerated. As the VAN was increased to 200 mg L^{-1}, a level that should inhibit most gram-positive bacteria, the HCB-dechlorination completely stopped. The reason could be that the gram-positive bacteria usually play the role of supplementing substrate to methanogenic bacteria, and once this supplementation is terminated, the methanogenic HCB-dechlorinator could no longer dechlorinate HCB. In addition, apart from methanogenic HCB-dechlorinators, it is possible that there were other naturally existing dechlorinators in the sediment of the Hua Lum Poo Canal, and they were most likely the gram-positive microbes that were able to tolerate VAN to some certain degree. Swenson et al. [17] reported that some gram-positive bacteria, such as the *Leuconostoc*, *Pediococcus*, and *Lactobacillus* species, were essentially resistant to vancomycin; however, they might be inactivated by the high VAN dose of 200 mg L^{-1}.

3.5. Kinetics of HCB-dechlorination

To evaluate the microbial dechlorination ability and HCB toxicity, the HCB dose was increased from 2 to 10, 40, 100, and 200 mg L^{-1}, which were equivalent to 43.8, 175.2, 438.0, 876.0 mg kg^{-1} dry solids for H1, and 63.1, 252.4, 630.9, 1261.8 mg kg^{-1} dry solids for H2, respectively. Surprisingly, it was found that HCB was still dechlorinated at the very high concentration of 200 mg L^{-1} with a minor retardation as illustrated in Table 4. The lag phase and appearance time for dechlorination intermediates including QCB, 1,2,3,5-TeCB, and 1,3,5-TCB were almost similar for all HCB dosages. The only difference was the time required to completely remove HCB, which became longer as the HCB increased. It is important to mention that the appearance times of 1,2,3,5-TeCB, and 1,3,5-TCB in the experiment with 2 mg L^{-1} HCB using the sediment slurry from H2 were different from those of the control set (no addition) in Table 3 even though they were tested under similar conditions with the same sediment sample. This was possibly due to the effect of the storage time at 4 °C in the cold storage. The sediment used in this part was kept for one week after sampling whereas those used in Table 3 was kept for two months. It implies that the QCB and 1,2,3,5-TeCB dechlorinators might be somehow affected during the storage at 4 °C for a long period of time. The degradation pathway of all HCB dosages still followed the major pathway to 1,3,5-TCB. The initial dechlorination rate was determined during the most active dechlorination period, i.e., HCB decreased sharply, as shown in Fig. 1. The relationship between the initial dechlorination rate and the HCB concentration is shown in Fig. 5. The dechlorination rate increased exponentially as the HCB concentration increased and finally reached a plateau where a further increase in HCB did not promote the rate. This pattern was similar to the enzymatic catalytic behavior that could be quantitatively explained by Michaelis–Menten kinetics. With sufficient nutrients and no inhibition effect as in this study, the dechlorination rate of HCB via a co-metabolization could be described under the influence of HCB and organic substrate concentrations as shown in Eq. (1) [16]:

$$\frac{d[HCB]}{dt} = -\left(\frac{k_m X[HCB]}{K_{HCB} + [HCB]}\right)\left(\frac{S}{K_S + S}\right) \quad (1)$$

where k_m is the maximum dechlorination rate of HCB per unit biomass; K_{HCB} and K_S are the half-saturation constants regarding on HCB and organic substrate, respectively; X is the HCB-dechlorinator intensity; and S is the organic substrate concentration. Since the organic substrates (S) available for biodegradation as represented by the COD values were much higher than HCB concentration and the biomass (X) growth under anaerobic condition was considerably very slow, these two parameters can be considered constant

Table 4
HCB-dechlorination under various HCB concentrations.

Site	HCB (mg L^{-1})	Appearance time (days)			
		QCB	1,2,3,5-TeCB	1,3,5-TCB	Complete HCB-dechlorination time (days)
H1	2	14	14	14	70
	10	14	28	28	175
	40	14	28	35	>175
	100	14	28	42	>175
	200	21	28	49	>175
H2	2	14	21	28	70
	10	14	21	28	175
	40	14	21	28	175
	100	14	28	28	175
	200	14	28	35	>175

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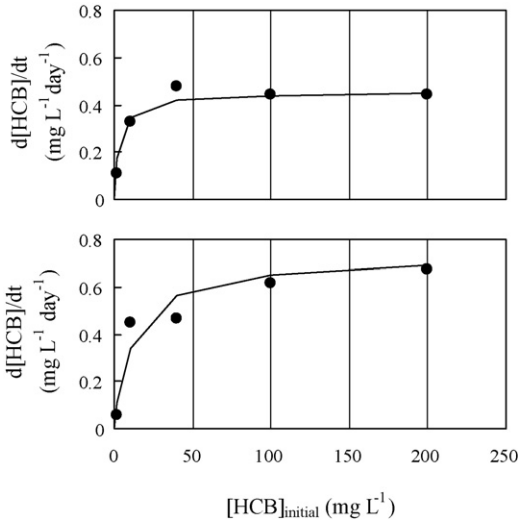

Fig. 5. Effect of HCB concentration on dechlorination rate (lines are model predictions based on the Michaelis–Menten kinetics).

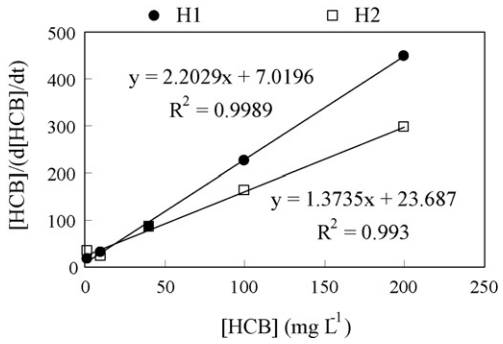

Fig. 6. Hanes linearization for Fig. 5.

during the initial stage; then, Eq. (1) can be simplified to:

$$\frac{d[HCB]}{dt} = -\left(\frac{k'_m[HCB]}{K_{HCB} + [HCB]}\right) \quad (2)$$

where k'_m is an apparent maximum dechlorination rate of HCB. The dechlorination rate then becomes first order with respect to HCB at low concentration and zero order at high concentration. Although many researchers working with HCB-dechlorination have successfully described their kinetic data by using either the first-order reaction [5,7] or the zero-order reaction [18], others decided to use a more realistic Michaelis–Menten model. Pavlostathis and Prytula [16] dechlorinated HCB by using enriched cultures supplementing with essential minerals, vitamins, yeast extract, and glucose, and found the dechlorination to follow the Michaelis–Menten model with the k'_m and K_{HCB} of 0.015 ± 0.001 mg L^{-1} day^{-1} and 0.024 ± 0.003 mg L^{-1}, respectively. Further analysis using the Hanes linearization method [19] as illustrated in Eq. (3) and Fig. 6 found the k'_m, K_{HCB}, and R^2 to be 0.45 mg L^{-1} day^{-1}, 3.2 mg L^{-1}, and 0.993 for H1 and 0.73 mg L^{-1} day^{-1}, 17.2 mg L^{-1}, and 0.999 for H2, respectively.

$$\frac{[HCB]}{(d[HCB]/dt)} = \frac{[HCB]}{k'_m} + \frac{K_{HCB}}{k'_m} \quad (3)$$

The k'_m and K_{HCB} obtained from this study were 30–50 and 178–480 times, respectively, higher than the values reported by Pavlostathis and Prytula [16]. The differences might be due to several factors such as HCB contamination and acclimation level, complexity of microbial consortium, and environmental factors particularly temperature as discussed previously. In addition, it is surprising to obtain significant differences in k'_m and K_{HCB} between H1 and H2 since H2 is only approximately 50 m downstream from H1. From field survey, it was found that H1 was mainly contaminated with industrial wastewater, whereas H2 was located in the congested low-income community; hence, it was also polluted with improperly treated domestic wastewater. Organic matter and nutrients from domestic wastewater might promote microbial activity in the direction of enhancing HCB-dechlorination.

4. Conclusions

The reductive dechlorination of HCB by indigenous sediment microbes under anaerobic condition was intensively investigated in this study. The indigenous microbes in the sediments collected from the Hua Lum Poo Canal could dechlorinate HCB effectively without acclimation and extra nourishment. Temperature significantly affected the HCB-dechlorination with the optimum range between 30 and 35 °C. Hence, HCB might not be a persistent organic pollutant in the moderate temperature environment like those in the tropical zone. The SRB, DN, and acetogens did not directly engage in the dechlorination of HCB; however, the SRB interfered with the dechlorination of QCB to 1,2,3,5-TeCB and 1,2,3,5-TeCB to 1,3,5-TCB. Methanogens and some VAN-resistant species of gram-positive bacteria were most likely the candidates for HCB-dechlorination. The HCB-dechlorination behavior could be sufficiently explained by the Michaelis–Menten kinetics with the k'_m and K_{HCB} between 0.45–0.73 mg L^{-1} day^{-1} and 3.2–17.2 mg L^{-1}, respectively.

Acknowledgements

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HEXACHLOROBENZENE DECHLORINATION BY ENRICHED MIXED CULTURES FROM THAI CANAL

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SUMMARY

After collecting and analyzing the sediments from 10 sites along the Hua-Lum-Poo Canal located in Samut Prakarn, a province of Thailand, hexachlorobenzene (HCB) and 1,3,5-trichlorobenzene (1,3,5-TCB) were found in four sediment samples. Due to 1,3,5-TCB has never been made commercially for industrial and agricultural usage, most of it was transformed from highly chlorinated benzenes. Therefore, it suggested that HCB has been dechlorinated to 1,3,5-TCB in the natural sediment of Hua-Lum-Poo Canal. This study was devoted to investigate the reductive dechlorination of HCB by the anaerobic microorganisms in canal sediments. Indigenous microorganisms from 10 canal sediments were collected and applied to dechlorination experiments. By using anaerobic microbes from yeast extract pre-enriched natural sediment-water slurries, every set of microorganisms initiated HCB dechlorination after 14 days of incubation and completed the process in a period of 28 ~ 42 days. In the sets without yeast extract pre-enrichment, the microorganisms also showed dechlorination activity after 14 ~ 28 days of cultivation. Combining the results, it showed that after 7-days of pre-anoxic and enriched treatment, canal microbes were able to develop the HCB dechlorination activity and completely dechlorinate HCB within 6 weeks. This indicates that the HCB dechlorinating microorganisms were prevalent in the sediments along the Hua-Lum-Poo Canal and possibly to remove HCB contamination without any additional nutrient.

KEYWORDS

Dechlorination, Hexachlorobenzene, Natural Sediment, anaerobic microorganisms

INTRODUCTION

After decades of uncontrolled releases, hexachlorobenzene (HCB) has been detected in air, water and sediment around industrial sites and farming area [1, 2]. It was produced and used as fungicide as well as an industrial synthetic material and generated as a byproduct of solvents and chlorinated chemical manufactures [3, 4]. Nowadays, HCB is well known as a priority pollutant in critical environment because of their specific properties such as carcinogenicity, recalcitrance and bioaccumulation. Thus, the removal of HCB from contaminated environments has become more significant. Clean-up methods such as chemical processes had been investigated [5, 6]. However, in consideration of lesser expense and environmental impact, biological treatment was necessarily advocated. The biodegradation of chlorinated benzenes by aerobic microbes could only deal with less-chlorinated ones, including monochlorobenzene (MCB), dichlorobenzenes (DCBs) and trichlorobenzenes (TCBs) [7, 8]. For highly chlorinated compounds, with the bulky chlorine atoms surrounding the benzene ring like pentachlorobenzene (QCB) and HCB, the aerobic biodegradation was seemingly completely inhibited.

The removal of chlorines from HCB and other highly chlorinated aromatics was considered as the first step to decompose these compounds and it was indeed effective through anaerobic dechlorination by using acclimated or enriched microorganisms [8, 9, 10]. This study was therefore dedicated to assess the dechlorination of HCB by the indigenous microbes collected from Hua-Lum-Poo Canal sediments, where some of the sediments were contaminated by HCB. In order to evaluate the dechlorination possibility, sediment microbes were collected from 10 sites along the entire canal, enriched and introduced to HCB dechlorination experiments. The degradation of HCB was studied by measuring the transformation of HCB to less-chlorinated compounds under reductive dechlorination.

MATERIALS AND METHODS

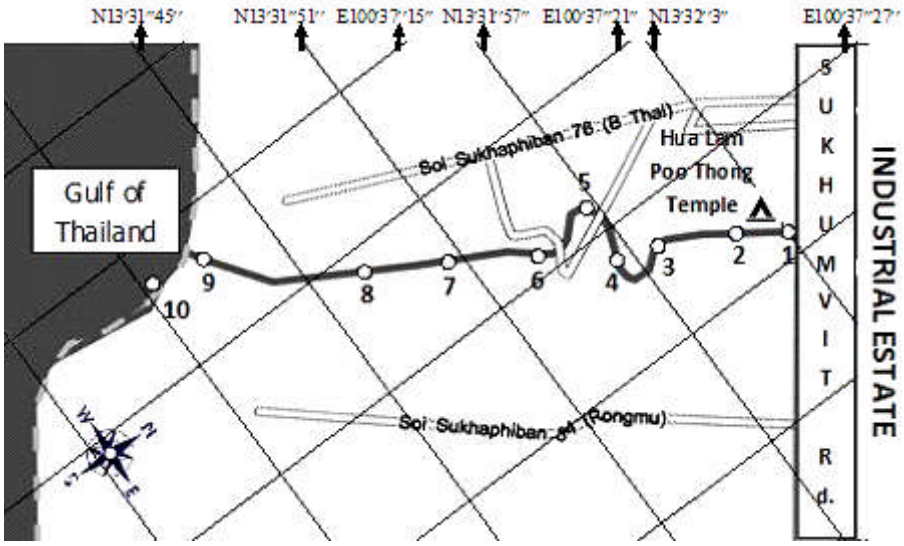

FIGURE 1 - Sediment and river water sampling sites along Hua Lam Poo Canal and seashore as marked from 1 to 10.

SEDIMENTS AND CHEMICALS

Sediments and river waters were collected from 10 sites (S1 to S10, from wastewater discharging site to estuarine) along Hua-Lum-Poo Canal, Bangpoo, Thailand as shown in Fig 1. After the sediment dug up, the upper 5 cm of sediments were carefully removed, and the remains were packed in plastic bags and kept in 4°C.

Chlorinated benzene congeners (CBs) including MCB, 1,2-, 1,3- and 1,4-DCB, 1,2,3-, 1,2,4- and 1,3,5-TCB, 1,2,3,4-, 1,2,3,5- and 1,2,4,5-Tetrachlorobenzene (TeCB), PCB and HCB were purchased from Riedel-de Haen Chemical Co. (Seelze, Germany).

MEDIA

Sediment slurry preparation

400 ml of sediment slurry and 400 ml of river water were placed in a screw-capped serum bottle (1L), and vigorously shaken by hand for 2 minutes. After the solid part settled down, 50 ml of the liquid part was withdrawn. And this sediment slurry (SS) was then transferred into an alumina-capped serum bottle.

Synthetic mineral medium

A synthetic mineral medium (MM) was also established, consisting of NH_4Cl, 2.7 g/L; $MgCl_2 \cdot 6H_2O$, 0.1 g/L; $CaCl_2 \cdot 2\ H_2O$, 0.1 g/L; $FeCl_2 \cdot 4H_2O$, 0.02 g/L; K_2HPO_4, 0.27 g/L; KH_2PO_4, 0.35 g/L, according to a previous study [9]. In some experimental sets, yeast extract (YE) was used as nutrient supplement with a concentration of 5 g/L in mineral medium or sediment slurry.

PREPARATION OF INOCULUM

Yeast extract (YE) enriched mixed cultures were used as inoculum. For the preparation of inoculums, the collected sediment was mixed with river water (50ml:50ml) and 1 g of YE, then placed into a serum bottle, shaken by hand for 1 minute and sealed. Samples were stored for 1 week in the dark at room temperature.

DECHLORINATION EXPERIMENT

After shaking the YE-enriched bottles, 5ml of YE enriched mixed cultures were withdrawn and injected into serum bottles which contained 45 mL of the medium and 2 mg/L of HCB, and then incubated at room temperature. The dechlorination procedures were described in detail from previous researches [11, 12]. At designated intervals, 2 ml of the incubated culture sample was taken from the serum bottle by a syringe.

ANALYSIS

The culture sample was extracted three times by *n*-hexane, and analyzed by a gas chromatography equipped with an ECD (Hewlett Packard 4890, Rockville, MD) and a capillary column of DB-5 fused silica. The oven

temperature was maintained at 80°C for 5 min, raised to 120°C at 5°C /min, maintained for 2 min, and then raised again at 5°C/min to the final temperature of 200°C, which was held for 5 min. The temperatures of the injector and the detector were set at 280°C and 300°C, respectively. Nitrogen and Helium gas were employed as the make-up and the carrier gas, respectively. The linear velocity was 16 cm/s and the split ratio was kept at 10:1.

RESULTS AND DISCUSSION

CBs IN CANAL SEDIMENTS

All ten sediments were first extracted to investigate the chlorinated benzenes contamination of the canal. The results showed that the first four sites close to the discharging location (S2to S5) were contaminated by HCB in a range of 0.182 to 1.250 ppm (Table 1). Surprisingly, 1,3,5-TCB was found in two of these HCB-contaminated sediments. Unlike HCB, 1,3,5-TCB was out of any industrial and farming usage and no published research suggested this compound could be an intermediate or an impurity during chemical processes [13]. However, many studies reported that 1,3,5-TCB was a very important final product in HCB dechlorination following the major pathway as described by Fathepure et al.; Holliger et al. and Chang et al. [14, 15, 16]. This finding provided a piece of evidence that HCB could be dechlorinated in natural sediment environment of Hua-Lum-Poo Canal.

TABLE 1 - The concentration of HCB and 1,3,5-TCB in Hua-Lum-Poo Canal sediments.

Sampling site	HCB (ppm)	1.3.5-TCB (ppm)
S2	0.182	ND
S3	1.250	0.238
S4	0.261	0.160
S5	0.153	ND

HCB DECHLORINATION IN NATURAL SEDIMENT SLURRIES

After 1 week of YE pre-enrichment, anaerobic mixed cultures from all ten sites has showed HCB-dechlorination ability in sediment slurry without yeast extract (Table 2). Eight of them dechlorinated HCB after 14 days of incubation and the other two have a lag phase of 28 days. However, HCB dechlorination was completed before 42 days of incubation. Within the findings of the HCB-dechlorination by river sediment microorganisms from southern Taiwan [17], only one from Erh-Jen River sets and one from Ho-Tsin River sets were able to dechlorinate HCB in a natural sediment water media, and the dechlorination was never completed (Table 2). It showed the superior dechlorination ability of Hua-Lum-Poo Canal microbes. Under similar conditions with Taiwan studies, using Hua-Lum-Poo SS media, all enriched cultures initiated and completed the dechlorination within a much shorter period.

The dechlorinating intermediates found in HCB-dechlorination processes were QCBs, 1,2,3,5-/1,2,4,5-TeCB (co-eluting congeners) and 1,3,5-TCB, nevertheless the last was the only product remained. As shown in Figure 2, HCB dechlorination by Site 4 microbes produced QCB and 1,2,3,5-/1,2,4,5-TeCB on day 24, and successively to 1,3,5-CB. The dechlorination pattern by Hua-Lum-Poo Canal microbes followed the major pathway: HCB → PCB → 1,2,3,5-/1,2,4,5-TeCB → 1,3,5-TCB, as described from previous studies [11, 17]. As Chen et al. suggested, the untamed microbes in Taiwan favorably dechlorinated HCB through the dechlorination reactions with larger amount of energy released and also with greater difference in chromatographic properties [17]. Therefore, microorganisms from mild contaminated sites also dechlorinated HCB by following the energy releasing favorable pathway.

HCB DECHLORINATION IN SEDIMENT SLURRIES SUPPLEMENTED WITH YEAST EXTRACT

With the supplement of yeast extract, organic contents from the cultural media increased and helped the YE-enriched microbes to be more vigorous, quickly turning the serum bottle condition into strictly anaerobic. Table 3 showed that all ten canal anaerobic mixed cultures could dechlorinate HCB with a lag phase of 14 days, two of them has a shorter lag phase than the set without YE (Table 2). This is similar to the finding of Chen et al., in the investigation of the HCB dechlorination ability of untamed sediment anaerobic microbes in Erh-Jen River and Ho-Tsin River [17]. All microbial consortia from Erh-Jen River, Ho-Tsin River and Hua-Lum-Poo Canal sediments showed the enhancement of HCB-dechlorinating activity by YE supplement (Table 3). It suggested that HCB-dechlorination microorganisms is widespread over Taiwan rivers and Thai canal sediments. And the

environmental remediation of HCB pollution is possible with the potential of anaerobic microorganisms that originated in the contaminated sites. However, a noble HCB-dechlorination happened in the mixed cultures from Hua-Lum-Poo Canal. With YE (Table 3) or without YE (Table 2), both sets of microbes dechlorinated HCB faster than the sets from Taiwan rivers. The lag phase and dechlorination period (from the beginning up to the end of dechlorination) of Thai canal were 2 to 4 times shorter than Taiwan river sets.

TABLE 2 - HCB Dechlorination by sediment microorganisms from Hua-Lum-Poo Canal sediments (S1 ~ S10), Erh-Jen River sediments (E1,E2) and Ho-Tsin River sediments (H1, H2) by using sediment slurry (SS) without yeast extract as media.

Inoculum source[a]	Medium	Yeast extract amendment (g/L)	Lag phase (day)	Dechlorination completing day	HCB degradation ratio (%)	Final Product
S1	SS[b]	0	14	28	100	1,3,5-TCB
S2	SS	0	14	42	100	1,3,5-TCB
S3	SS	0	28	42	100	1,3,5-TCB
S4	SS	0	14	28	100	1,3,5-TCB
S5	SS	0	14	42	100	1,3,5-TCB
S6	SS	0	14	42	100	1,3,5-TCB
S7	SS	0	28	42	100	1,3,5-TCB
S8	SS	0	14	42	100	1,3,5-TCB
S9	SS	0	14	42	100	1,3,5-TCB
S10	SS	0	14	42	100	1,3,5-TCB
E1[c]	SS	0	ND[d]	ND	0	ND
E2	SS	0	90	NC[e]	59	1,3,5-TCB
H1	SS	0	ND	ND	0	ND
H2	SS	0	75	NC	62	1,3,5-TCB

[a]Inoculum of S1 ~ S10 from Hua-Lum-Poo Canal enriched with yeast extract for 1 week; inoculum of E1 ~ H2 from Erh-Jen River and Ho-Tsin River pre-cultured under anaerobic condition for 2 weeks without yeast extract.

[b]SS: Sediment slurry, mixed sediment and river water (1:1), then withdrawing the supernatant.

[c]Data of E1 ~ H2 taken from "Microbial dechlorination of hexachlorobenzene by untamed sediment microorganisms in Taiwan. Chen et al., Practice Periodical of Hazardous, Toxic, and Radioactive Waste Management" [17].

[d]ND: No dechlorination after 150 days of incubation period.

[e]NC: Dechlorination was not completed after 150 days of incubation period.

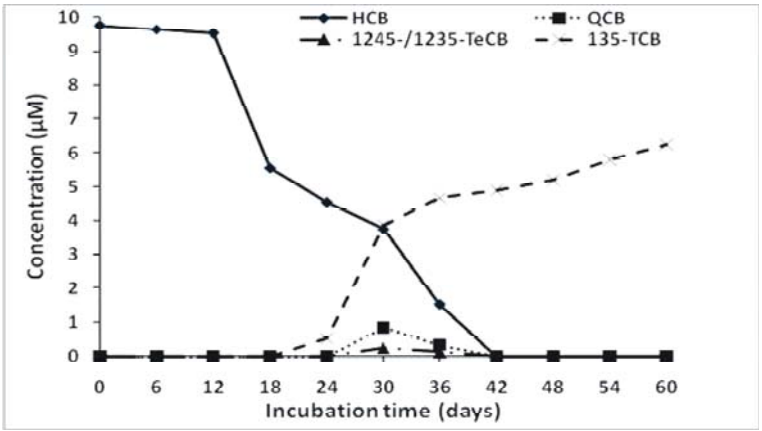

FIGURE 2 - HCB dechlorination by site 4 (S4) indigenous microorganisms from canal sediments with sediment slurry (SS) as media.

TABLE 3 - HCB dechlorination by sediment microorganisms from Hua-Lum-Poo Canal sediments (S1 ~ S10), Erh-Jen River sediments (E1, E2) and Ho-Tsin River sediments (H1, H2) by using sediment slurry (SS) with yeast extract as media.

Inoculum source[a]	Medium	Yeast extract amendment (g/L)	Lag phase (day)	Dechlorination completing day	Final Product
S1	SS[b]	5	14	28	1,3,5-TCB
S2	SS	5	14	28	1,3,5-TCB
S3	SS	5	14	42	1,3,5-TCB
S4	SS	5	14	42	1,3,5-TCB
S5	SS	5	14	42	1,3,5-TCB
S6	SS	5	14	42	1,3,5-TCB
S7	SS	5	14	28	1,3,5-TCB
S8	SS	5	14	28	1,3,5-TCB
S9	SS	5	14	42	1,3,5-TCB
S10	SS	5	14	42	1,3,5-TCB
E1[c]	SS	5	60	110	1,3,5-TCB
E2	SS	5	30	90	1,3,5-TCB
H1	SS	5	60	110	1,3,5-TCB
H2	SS	5	30	90	1,3,5-TCB

[a]Inoculum of S1 ~ S10 from Hua-Lum-Poo Canal enriched with yeast extract for 1 week; inoculum of E1 ~ H2 from Erh-Jen River and Ho-Tsin River pre-cultured under anaerobic condition for 2 weeks without yeast extract.
[b]SS: Sediment slurry, mixed sediment and river water (1:1), then withdrawing the supernatant.
[c]Data of E1 ~ H2 taken from "Microbial dechlorination of hexachlorobenzene by untamed sediment microorganisms in Taiwan. Chen et al., Practice Periodical of Hazardous, Toxic, and Radioactive Waste Management" [17].

HCB DECHLORINATION IN SYNTHETIC MINERAL MEDIA SUPPLEMENTED WITH YEAST EXTRACT

Using synthetic mineral medium (MM) amended with 5 g/L of yeast extract as media, HCB dechlorination (Table 4) looked no significant difference from the sets using sediment slurry added with YE (Table 3). Almost every bottle showed the same lag phase and dechlorination period, with only one exception of site 9 (S9). The dechlorination period of MM-YE bottles was 28 days shorter than SS-YE bottles. The efficiency of HCB-dechlorination in MM-YE by Thai canal microbes was also higher than Taiwan river microbes. This result revealed that HCB dechlorination in Thai canal is effective not only because the canal sediment slurry was more nutritious, but also from the powerful dechlorination ability of canal indigenous microorganisms.

CONCLUSIONS

For the removal of chlorinated aromatic compounds like hexachlorobenzene, pentachlorophenol, polychlorinated biphenyls and dioxins in contaminated environments, the strategy of mineralization by aerobic microorganisms is not available. Because of the concentration of these compounds in natural environment was always too low to initiate the aerobic microbial degradation. Furthermore, the chlorine atoms on the benzene ring will block the attack on the ring. Microbial reductive dechlorination was a kind of co-metabolism, dechlorinating anaerobic consortia degraded the chlorinated compounds only for energy harvesting. This kind of reaction can be activated under very low concentration of the target compound. Therefore, nowadays dechlorination was thought to be the only one way in the remediation of chlorinated aromatics pollution. In this study, the dechlorination ability and dechlorinating activity of anaerobic microorganisms in Thai canal were well evaluated. YE pre-enriched microbes from all 10 sites along whole Hau-Lum-Poo Canal could dechlorinate HCB within 42 days of

incubation. The results strongly suggested that the HCB degradation in natural Thai Canal was possible, and this kind of biodegradation was valuable for the remediation of chlorinated aromatic contamination in Thailand.

TABLE 4 - HCB Dechlorination by sediment microorganisms from Hua-Lum-Poo Canal sediments (S1 ~ S10), Erh-Jen River sediments (E1,E2) and Ho-Tsin River sediments (H1, H2) by using synthetic mineral medium (MM) with yeast extract amendment as media.

Inoculum source[a]	Medium	Yeast extract amendment (g/L)	Lag phase (day)	Dechlorination completing day	Final Product
S1	MM[b]	5	14	28	1,3,5-TCB
S2	MM	5	14	28	1,3,5-TCB
S3	MM	5	14	42	1,3,5-TCB
S4	MM	5	14	42	1,3,5-TCB
S5	MM	5	14	42	1,3,5-TCB
S6	MM	5	14	42	1,3,5-TCB
S7	MM	5	14	28	1,3,5-TCB
S8	MM	5	14	28	1,3,5-TCB
S9	MM	5	14	28	1,3,5-TCB
S10	MM	5	14	42	1,3,5-TCB
E1[c]	MM	5	45	90	1,3,5-TCB
E2	MM	5	30	60	1,3,5-TCB
H1	MM	5	45	90	1,3,5-TCB
H2	MM	5	30	75	1,3,5-TCB

[a] Inoculum of S1 ~ S10 from Hua-Lum-Poo Canal enriched with yeast extract for 1 week; inoculum of E1 ~ H2 from Erh-Jen River and Ho-Tsin River pre-cultured under anaerobic condition for 2 weeks without yeast extract.
[b] SS: Sediment slurry, mixed sediment and river water (1:1), then withdrawing the supernatant.
[c] Data of E1 ~ H2 taken from "Microbial dechlorination of hexachlorobenzene by untamed sediment microorganisms in Taiwan. Chen et al., Practice Periodical of Hazardous, Toxic, and Radioactive Waste Management" [17].

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ผลของสารให้อิเลคตรอนต่อกระบวนการลดคลอรีนของ 2,3,4-ไตรคลอโรไบฟีนิลโดยจุลชีพ

Effect of Electron Donor on Microbial Dechlorination of 2,3,4-Trichlorobiphenyl

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บทคัดย่อ

งานวิจัยชิ้นนี้ศึกษาถึงการย่อยสลาย 2,3,4-ไตรคลอโรไบฟีนิลโดยกลุ่มจุลชีพแบบไร้อากาศในตะกอนลำน้ำจากแม่น้ำเออร์เจนทางตอนใต้ของประเทศไต้หวันและจากคลองข้างถนนตำหรุ-บางพลีในจังหวัดสมุทรปราการ ประเทศไทย โดยทำการเติมสารสกัดยีสต์และ/หรือสารให้อิเล็กตรอนคือ ไพรูเวท อะซิเทท หรือแลคเทท ผลการศึกษาในชุดควบคุมที่ไม่มีการเติมสารให้อิเลคตรอนและสารสกัดยีสต์พบว่าจุลชีพในน้ำตะกอนจากคลองข้างถนนตำหรุ-บางพลีมีความสามารถในการลดคลอรีนของ 2,3,4-ไตรคลอโรไบฟีนิลได้โดยมีช่วงหน่วง 10 สัปดาห์ ในขณะที่น้ำตะกอนจากแม่น้ำเออร์เจนไม่สามารถทำได้ คาดว่าเป็นผลมาจากความแตกต่างในด้านความหลากหลายของจุลชีพที่มีอยู่ในตะกอนตามธรรมชาติจากแหล่งน้ำทั้งสองซึ่งอยู่ในพื้นที่ที่มีภูมิอากาศแตกต่างกัน การเติมไพรูเวทและแลคเททลงในน้ำตะกอนจากคลองข้างถนนตำหรุ-บางพลีช่วยเร่งการย่อยสลายของ 2,3,4-ไตรคลอโรไบฟีนิลได้โดยช่วงหน่วงลดลงจาก 10 สัปดาห์เป็น 4-6 สัปดาห์ ในขณะที่อะซิเททไม่มีผลที่เด่นชัดต่อการลดคลอรีน ชี้ให้เห็นว่าแบคทีเรียกลุ่มสร้างกรดที่ใช้ไพรูเวทและแลคเททเป็นสารอาหารน่าจะมีส่วนร่วมโดยตรงในการลดคลอรีนของ 2,3,4-ไตรคลอโรไบฟีนิล ในขณะที่แบคทีเรียกลุ่มสร้างมีเทนซึ่งใช้อะซิเททไม่ได้เป็นจุลชีพหลักที่จำกัดการลดคลอรีนในน้ำตะกอนจากคลองข้างถนนตำหรุ-บางพลี ในทำนองกลับกันการเติมสารสกัดยีสต์และสารให้อิเลคตรอนไม่สามารถช่วยให้เกิดการย่อยสลาย 2,3,4-ไตรคลอโรไบฟีนิลในน้ำตะกอนจากแม่น้ำเออร์เจนได้เลยถึงแม้ว่าจะมีการเติมซ้ำทุกๆ 2 สัปดาห์ก็ตาม แสดงว่าจุลชีพที่เกี่ยวข้องกับการย่อยสลาย 2,3,4-ไตรคลอโรไบฟีนิลจำเป็นต้องใช้เวลานานในการเจริญเติบโตและสร้างเอนไซม์ที่เกี่ยวข้อง

คำสำคัญ: พีซีบี; สารให้อิเล็กตรอน; ตะกอนดินเหลว; การลดคลอรีน

Abstract

This research studied the dechlorination of 2,3,4-trichlorobiphenyl by anaerobic sediment microbes from Erh-Jen River in southern of Taiwan and from a canal along the Tumhru-Bangplee Road in Samuth Prakarn Province of Thailand by amending the sediment slurries with yeast extract and/or electron donor either pyruvate, lactate or acetate. In the control experiment in which neither yeast extract nor electron donor was added, the sediment slurry from the Tumhur-Bangplee Road could declorinate 2,3,4-trichlorobiphenyl with the lag phase of 10 weeks whereas those from Erh-Jen River could not. This should be due to the difference in the microbial diversity/consortium of the natural sediments between these two streams which are located in different climate zones. Pyruvate and lactate could enhance the dechlorination of 2,3,4-trichlorobiphenyl in the canal sediment slurry, i.e., the lag phase was reduced from 10 weeks to 4-6 weeks whereas acetate did not have any significant impact on the dechlorination process. This indicates that the acid-forming bacteria which can use pyruvate and lactate as their substrate might directly involve in the dechlorination of 2,3,4-trichlorobiphenyl. On the other hand, the methane-forming bacteria which use acetate as their substrate might not be the principal microorganisms controlling the dechlorination process in this canal sediment. There was no dehlorination happened in the sediment slurry of Erh-Jen River even with multiple supplements every 2 weeks indicating that the 2,3,4-trichlorobiphenyl dechlorinators required long period of time to growth and produce related enzymes.

Keywords: PCB; electron donor; sediment slurry; dechlorination

1. Introduction

Polychlorinated biphenyls (PCBs) are stable organic compounds which consist of 1-10 chlorine atoms in biphenyl rings and have the chemical formula of $C_{12}H_{10-n}Cl_n$ as shown in Figure 1. PCBs were first produced commercially in the United States around 1929, and since that time they have had a wide range of applications including uses as dielectrics in capacitors and transformers, as a petroleum additive, plasticizer, lubricant, and in carbonless copy paper. This extensive use has also led to widespread contamination of PCBs in the environment, causing great concern due to their extreme persistence, bioaccumulation, and potential adverse health effects. The use of PCBs in Thailand has been banned since 1975; however, PCBs are still detected in Thailand after 1975 due to improper handling and storage of used transformers and condensers and accidental leakage of PCBs [1]. Bacteria in soils and sediments under anaerobic condition can selectively remove chlorines from meta- and para-positions of the biphenyl ring, appearing to reduce the toxicity and bioaccumulation potential of residues. These chlorinations were limited by PCB concentration [2], position of chlorine atom, group of microorganism, electron donor and electron acceptor, and microbial inhibitor [3]. Therefore, this study aims to investigate the influence of electron donors on the PCBs dechlorination ability in order to understand the dechlorination mechanisms and activity of the microbial dechlorinating consortium.

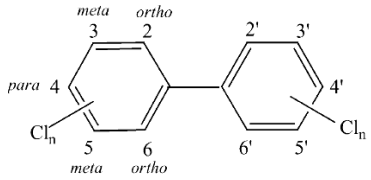

Figure 1. Structural formula of PCBs.

2. Materials and Methods

2.1 Sediment and Stream Water Samples

Sediments and stream waters were collected from the Erh-Jen River along Tainan and Kaohsiung Counties in Southern of Taiwan and from a canal along the Tunhru-Bangplee road which receiving discharge from small material recovery facilities in Samuth Prakarn Province in Thailand. The media (sediment slurry) were freshly prepared prior to each experiment by mixing sediment and stream water at the ratio of 1:1 (v/v), sieved to remove the particles larger than 0.7 mm, and kept in an alumina capped serum bottle.

2.2 Experimental Setup

2.2.1 Characterization of 2,3,4-CBp Dechlorination by Amendment with Nutrient and Electron Donor.

In this scenario, 50 ml of sediment slurry was transferred to a 100 ml serum bottle and capped with the alumina-cap. Each serum bottle was then added with yeast extract at the concentration of 5 g/l. Either pyruvate, acetate, or lactate was used as an electron donor source. Each organic compound was injected into the serum bottles to make the final concentration of 20 mM. 2,3,4-CBp was injected into all serum bottles to make the initial concentration of 2 ppm. All serum bottles were kept in the dark under local room temperature (either of Taiwan or Thailand). Residual PCBs, its intermediates and methane production were analyzed every 2 weeks.

2.2.2 Characterization of 2,3,4-CBp Dechlorination by Extended Nutrient Feeding.

In this scenario, only sediment slurries from the Erh-Jen River were used. Fifty ml of sediment slurry in the 100 ml serum bottle was inoculated with 2 g/l yeast extract and/or 1 g/l of electron donors. The nutrients were; however, added in two manners, i.e., as a single dose at the beginning and multi-dose every 2 weeks. Sediment slurry without external supplement was also prepared and served as the control. After that, 2,3,4-CBp was added into the serum bottles to make the initial concentration of 2 ppm then incubated in the dark under room conditions**.**

2.3 Extraction Methods

At predetermined time, 2 ml of liquid mixture was withdrawn from the serum bottle by a needle and injected into an extraction tube. Two ml of hexane and 0.2 ml of 6 N NaOH were added. The mixture was then shaken by hand for 100 times followed by ultra-sonicating for 10 minutes. The vial was centrifuged at 3,000 rpm for 10 minutes. The upper layer of extracting solvent was withdrawn as much as possible afterward and injected into a new tube. The remaining mixture was re-extracted for another two times following the similar procedures. At the third extraction, the upper layer

liquid was pulled out and filled the tube up to the 5-ml mark. A small amount of anhydrous Na_2SO_4 was added to remove moisture before GC analysis.

2.4 Sample Analysis

For PCBs analysis, a gas chromatograph equipped with an ECD (Agilent 6890N, USA) and a capillary column of DB-5 fused silica was used to analyze PCBs and their by-products. The oven temperature was maintained at 120°C for 2 min, raised to 225°C at 3°C/min, maintained for 3 min, and then raised again at 5°C/min to the final temperature of 270 °C, which was held for 11 min. The temperature of the injector and the detector was set at 280°C and 300°C, respectively. Nitrogen gas was employed as the carrier and the make-up gases, at the flow rate of 4 ml/min and the split ratio was kept at 10:1. Water characteristics were analyzed according to the procedures described in the Standard Methods [4].

3. Results and Discussion

3.1 Characteristics of Stream Water and Sediment Slurry

The pH and COD of the stream water and prepared sediment slurry from the canal in Thailand were 7.2 and 50 mg/l and 6.9 and 13,100 mg/l, respectively. It could be seen that the sediment slurry contained high organic content indicating a sufficient carbon source and suitable pH for microbial activity under anaerobic condition although most of the organics were in the solid form. The SS and VSS of the sediment slurry were 95,800 and 8,150 mg/l, respectively. Low VSS:SS ratio of 0.09 indicated that most of the solids were inert materials. Nonetheless, there were enough viable microorganisms in the sediment slurry to perform the anaerobic degradation activity since methane gas was produced rapidly and enormously. The characteristics of water and sediment slurry from Erh-Jen River in Taiwan in terms of pH and COD were not determined; however, enormous biogas was observed during the sediment sampling as well. Chen et al. [5] using the same sediment slurry from this site determined the soluble COD of the sediment slurry to be between 65 and 163 mg/l. Therefore, it is believed that the sediment slurries prepared from these two sites had sufficient and suitable nutrients and substrates for anaerobic degradation.

3.2 Effect of Nutrient and Electron Donor Supplement on 2,3,4-CBp Dechlorination

Even though the microorganisms in both sediment slurries were very active in term of anaerobic digestion, only the sediment slurry from the canal could dechlorinate 2,3,4-CBp as shown in Table 1. It is interesting to observe that the sediment slurry from the canal receiving discharge from material recovery facilities could dechlorinate 2,3,4-CBp even without any external supplements implying that the existing substrates and nutrients were already enough to support the dechlorination activity. As a result, the addition of yeast extract did not have any positive effects on the 2,3,4-CBp dechlorination. Pyruvate and lactate could notably improve the dechlorination ability by shortening the lag phase. Since both pyruvate and lactate are primary substrates to acidogenic bacteria, it implies that some acidogens might involve in the CBp-dechlorination process. On the other hand, acetate which promotes that methanogenic activity did not provide

Table 1. Effect of electron donor and nutrient supplement on 2,3,4-CBp dechlorination.

Site	Additive	Dechlorination	Product	Lag Phase (weeks)	Dechlorination completing week
Canal	Control	Yes	2,4-CBp	10	>16
	Yeast Extract	Yes	2,4-CBp	14	>16
	Pyruvate	Yes	2,4-CBp	6	>16
	Pyruvate+Yeast Extract	Yes	2,4-CBp	8	>16
	Acetate	Yes	2,4-CBp	14	>16
	Acetate+Yease Extract	Yes	2,4-CBp	12	>16
	Lactate	Yes	2,4-CBp	6	>16
	Lactate+Yeast Extract	Yes	2,4-CBp	4	>16
Erh-Jen	Control	No	-	-	-
	Yeast Extract	No	-	-	-
	Pyruvate	No	-	-	-
	Pyruvate+Yeast Extract	No	-	-	-
	Acetate	No	-	-	-
	Acetate+Yease Extract	No	-	-	-
	Lactate	No	-	-	-
	Lactate+Yeast Extract	No	-	-	-

any improvement in the dechlorination process. It indicated that the methanogenic bacteria might not directly involve in the dechlorination of 2,3,4-CBp. Only 2,4-CBp was detected as a product from 2,3,4-CBp dechlorination. This result indicated that the middle chlorine atom in the biphenyl ring was more susceptible to be removed than other positions similar to the study of Chen et al. [3]. Despite the rapid emerging capability in CBp dechlorination as illustrated by the short lag phase, 2 ppm of 2,3,4-CBp could not be completely degraded in 16 weeks. In contrast to the canal sediment slurry, the sediment slurry prepared from the Erh-Jen River could not dechlorinate 2,3,4-CBp in all scenarios even with the supplement of electron donor and nutrient. This led to an initial hypothesis that there was possibly no CBp-dechlorinator in the microbial matrix of this sediment or the room conditions in Taiwan were not suitable for CBp-dechlorinators. It is important to note that these two streams are in the areas with the difference climate classifications i.e., Thailand is in the tropical climate zone with steady temperature whereas Taiwan is in the temperate climate zone with low temperature during the winter. Hence the native microbes in the sediment of the canal in Thailand should be more diverse than those in the Erh-Jen River in Taiwan due to the effect of temperature. In addition, it was also found that this canal was more polluted than the Erh-Jen River in Taiwan. The abundant substrate and nutrient in the sediment of this canal might intensify the diversity and activity of the microbes to be further superior than those in the Erh-Jen River sediment.

Cho and Oh [6] indicated that microbiological populations were different from location to location and, hence, they may show different dechlorination ability. Chen et al. [7] found that the HCB-dechlorinators' activity was utmost between 30 and 40°C (typical temperature in Thailand) and became deteriorated as the temperature rose or dropped below this range. Furthermore, Chen et al. [5] were able to use mineral medium and yeast extract to stimulate the 2,3,4-CBp dechlorination ability of the sediment slurry of the Erh-Jen River. They found that the dechlorination could happen within 8 weeks of the incubation at 30°C and the only one intermediate product found was 2,4-CBp similar to this study in the case of sediment slurry from the canal. On the other hand, they found no dechlorination happen within 24 weeks in the control set without mineral medium and yeast extract supplement. This suggests that the 2,3,4-CBp-dechlorinating microorganisms actually existed in the sediment of Erh-Jen River; however, they required the right environments to promote their dechlorination activities.

3.3 Effect of Supplement Rate on 2,3,4-CBp Dechlorination

To verify whether the incompetent in substrate and nutrient uptake of the CBp-dechlorinators was actually the cause of no dechlorination activity observed in the sediment slurry prepared from the Erh-Jen River, the electron donor and nutrient was supplied continuously every two weeks. This supplement pattern ensured that the electron donor and nutrient would be present ceaselessly in the medium and, thus, sufficiently available for CBp-dechlorinators to take up and perform the dechlorination activities. However, the results still showed that 2,3,4-CBp could not be dechlorinated within 8 weeks of continuous supplement. As a result, it implies that the microbial consortiums in the sediment from Erh-Jen River might indeed contain no CBp-dechlorinator.

4. Conclusions

2,3,4-CBp could be dechlorinated to 2,4-CBp in the sediment slurry prepared from the sediment and water collected from the canal receiving the discharge from small material recovery facilities in Thailand without any supplement of electron donor or yeast extract. However, the sediment slurry of Erh-Jen River in Taiwan could not dechlorinate even with continuous supplement of substrate and nutrient suggesting different microbial diversity and activity between these two sediments. Temperature and substrate availability were most possible factors controlling the dechlorination process. Pyruvate and lactate could promote the dechlorination process in the canal sediment slurry implying that some species of acidogenic bacteria might involve in the 2,3,4-CBp dechlorination process whereas the methanogenic bacteria which use acetate as their substrate might not be the primary dechlorinator controlling the 2,3,4-CBp degradation. The result from the sediment slurry from Erh-Jen River also indicated that the development of the dechlorinator population and its activity took a long period of time.

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