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Natural Seawater Qualities Along the Gulf of Thailand Coast Areas for Plastic Biodegradation Test According to ASTM D6691

Jantana Panpran[1], Mirantee Deecharern[1], Nopphawan Srasaengta[1], Chanchai Kahapana[1], Suttida Kongjay[1], Sirorat Tungsatitporn[1], Anchana Pattanasupong[1]

1. Material Biodegradation Testing Laboratory, Material Properties Analysis and Development Centre, Thailand Institute of Scientific and Technological Research, Pathum Thani 12120, Thailand

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Abstract: ASTM D6691 could enhance competitiveness, reduce trade barriers, and ensure compliance with management regulations regarding plastic waste in each country. The natural seawater was collected from four provinces along the Gulf of Thailand coast three times a year to monitor the environment and qualities for biodegradation test method development in accordance with ASTM D6691, including the verification of test method validity. The data were analyzed using the Duncan Multiple Range Test. The parameters include ambient temperature, sea surface temperature, salinity, and dissolved oxygen were significantly differences ($p < 0.05$). The test method validity was assessed by utilizing reference materials and inoculating with the collected natural seawater. The criteria were to achieve a biodegradation percentage not less than 70% within 10 to 90 days and align with the requirements of ASTM D6691. It could be considered that the natural seawater from these areas were suitable to be applied as an inoculum for biodegradation test although some parameters may be changed depending on time period. The test method could identify the biodegradable properties of materials in the seawater, providing an alternative solution to solve the plastic waste problem.

Key words: ASTM D6691, Biodegradation, Cellulose chromatography grade, Plastic, Seawater.

1. Introduction

Plastic pollution has dominated social media feeds with powerful images in recent years, and their impact on the marine environment has become a center of research and public engagement [1]. Plastic manufacturing exceeds the world's ability to manage it when abandoned as waste [2]. As a result of

Corresponding author:
Anchana Pattanasupong, Thailand Institute of Scientific and Technological Research, Thailand. E-mail: anchana@tistr.or.th

overconsumption, an estimated 4.8–12.7 million metric tons of plastic waste enters to the environment every year mainly due to mismanaged waste [3]. Different affectations in animals have been reported: ingestion causing eating disorders, entanglement, and induced asphyxiation or other hurts due to plastic waste [4].

For many years, there are various different ways of handling plastic waste to reduce its impact on the environment and be more environmentally friendly such as recycling, degradable, oxo–biodegradable, and compostable. Consequently, they may contribute to the production of microplastics in the [5].

Biodegradation is an alternative method for environmental benefit. Several regulatory bodies (e.g. OECD, ASTM, ISO) issued standards to enable the assessment of the biodegradability of plastics in different environments such as soil, composting facilities, aquatic environment, and even marine [6]. Biodegradation could be formally defined as the mineralization of organic matter mediated by microorganisms, leading in aerobic conditions to CO_2 and H_2O, and to an increase in the biomass of microorganisms under a set of standardized incubation conditions representative of a given environmental compartment [7] such as polylactic acid (PLA) are designed to biodegrade in composting conditions [8].

This study presents the natural seawater qualities and stability that are suitable for plastic biodegradation test method development at the laboratory in accordance with ASTM D6691 “standard test method for determining aerobic biodegradation of plastic materials in the marine environment by a defined microbial consortium or natural seawater inoculum” [9] which was using cellulose chromatography grade as positive reference material. Therefore, the test method could be identified the biodegradable properties of materials in seawater, contributing to a significant decrease in the accumulation of plastic litter in the global ocean and also supporting implicated policies and industries.

2. Materials and Methods

2.1 Collection of natural seawater

Natural seawater samples were collected three times from November to December 2021, March to May 2022, and July to August 2022 from three areas of each province along the Gulf of Thailand coast, namely Chonburi, Rayong, Prachuap Khiri Khan, and Chumphon (Table 1 and Fig. 1). The collected seawater samples were determined for basic parameters such as ambient temperature, sea surface temperature, salinity, pH, dissolved oxygen and total bacteria count then used as an inoculum for evaluating the biodegradation test in accordance with ASTM D6691.

2.2 Development of biodegradation test in seawater

The biodegradation test was assessed under laboratory conditions through a respirometry system in accordance with ASTM D6691. The cellulose chromatography grade of approximately 0.25 ± 0.01 g/l was introduced into collected natural seawater enriched with inorganic nutrients 0.05 g/l of NH_4Cl and 0.1 g/l of $KH_2(PO_4)$ as a reference control and vessels containing only mixed seawater were used as blanks, followed by incubation at 30 ± 2˚C. The solutions were continuously stirred into the vessels within 10-90 days. Each treatment was performed in triplicate. The amount of biodegradation based on oxygen consumption was indicated as the ratio of the BOD to the chemical oxygen demand (COD) of the used test material.

Calculation of percentage biodegradation (OECD guideline for testing of chemicals. 1992) [10]

$$\text{Biodegradation (\%)} = \frac{\text{BOD}}{\text{COD}} \times 100$$

Biodegradation (%)	Percentage of biodegradation
BOD	BOD of test material (mg/mg)
COD	Chemical oxygen demand (mg/mg)

2.3 Statistical Analysis

The data were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. The statistical evaluation was conducted using SPSS 26.0 for Windows software (SPSS Inc, Chicago, Illinois). The results were presented as mean ± SD, with a statistically significant difference accepted at $P < 0.05$.

3. Results and Discussion

3.1 The natural seawater properties

The natural seawater was collected from provinces along the Gulf of Thailand coast, namely Chonburi, Rayong, Prachuap Khiri Khan, and Chumphon three times a year to determine the basic seawater quality and total bacteria count.

The mean results for the pH measurements in all collected seawater during the three time periods were significantly different ($p < 0.05$), whereas salinity were not significantly difference ($p > 0.05$). Dissolved

oxygen was significantly different ($p < 0.05$) in Chonburi, Rayong, and Prachuap Khiri Khan, moreover, ambient temperature, sea surface temperature, and total bacteria count were significantly different ($p < 0.05$) in Chonburi, Prachuap Khiri Khan and Chumphon (Table 2–5). Furthermore, the mean results over a year revealed that some parameters, such as ambient temperature, sea surface temperature, salinity, and dissolved oxygen, were significantly different ($p < 0.05$) in all collected seawater, whereas pH and total bacteria count were not ($p > 0.05$) (Table 6).

The Gulf of Thailand is also influenced by major rivers such as the Mae Klong River, the Tha Chin River, the Chao Phraya River, and the Bang Pakong River, as well as coastal activities, which may be another reason for variations in natural seawater properties from these areas due to evaporation, precipitation, river runoff, and seawater currents, [11-13] however dose not exceeding the seawater quality standards [14].

3.2 Biodegradation percentage of reference material

The cellulose chromatography grade was utilized as a reference material, and it was inoculated with collected natural seawater to evaluate biodegradation in seawater within 10–90 days in accordance with ASTM D6691, and the test validity requirement indicated that the biodegradation percentage of the reference material must be not less than 70%. The mean biodegradation percentages of reference material in collected natural seawater at different time periods from Chonburi and Chumphon were 76.66–91.42 and 76.94–86.75, respectively which were significantly different ($p < 0.05$) whereas Rayong and Prachuap Khiri Khan were 83.22–90.74 and 77.46–82.53, respectively which were not significantly different ($p > 0.05$) (Table 7). Furthermore, the biodegradation percentages of reference material for a year were 78.77–85.98 (Fig. 2). The results may be affected by environmental factors such as temperature and microorganisms in seawater at different time periods during the year and different areas, which lead to differences in degradation performance of the same material [15-16] and were similar to the report from BiologiQ, Inc., 2018 [17] that the biodegradation percentages of reference material were not less than 70 and could be passed the test validity requirement criteria.

4. Conclusion

Properties of natural seawater collected from each source did not different effect the biodegradation of the reference materials. Therefore, natural seawater from these areas is considered suitable as a substrate for seawater biodegradation testing in accordance with ASTM D6691.

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Table 1 Natural seawater collected areas

Province	Code	Details	Geographic coordinate	
			Latitude	Longitude
Chonburi	CB 1	Saen Suk Subdistrict, Mueang Chonburi District	13.30418	100.90194
	CB 2	Thung Sukhla Subdistrict, Si Racha District	13.12362	100.89759
	CB 3	Naklua Subdistrict, Banglamung District	12.97511	100.89683
Rayong	RY 1	Phla Subdistrict, Ban Chang District	12.66509	101.04643
	RY 2	Kram Subdistrict, Klaeng District	12.64905	101.62057
	RY 3	Neunkho Subdistrict, Klaeng District	12.69614	101.70121
Prachuap Khiri Khan	PCH 1	Sam Roi Yod Subdistrict, Sam Roi Yod District	12.24327	99.98411
	PCH 2	Ao Noi Subdistrict, Mueang Prachuap Khiri Khan District	11.84949	99.82567
	PCH 3	Huai Yang Subdistrict, Thap Sakae District	11.63576	99.70340
Chumphon	CHP 1	Saphli Subdistrict, Pathio District	10.57118	99.27483
	CHP 2	Hat Sai Ri Subdistrict, Mueang Chumphon District	10.35837	99.26628
	CHP 3	Suantang Subdistrict, Lamae District	9.74346	99.14528

Table 2 Natural seawater properties collected from Chonburi province at different time periods

Parameters	Collection period		
	A	B	C
1. Ambient temperature (ºC)	30.20±0.52^{a}	35.70±3.24^{b}	35.97±2.47^{b}
2. Sea surface temperature (ºC)	31.67±0.58^{a}	32.00±1.00^{a}	32.40±0.69^{a}
3. Salinity (ppt)	30.00±0.00^{a}	30.00±0.00^{a}	29.67±0.58^{a}
4. pH	7.23±0.02^{a}	8.00±0.02^{b}	7.92±0.09^{b}
5. Dissolved oxygen (mg/L)	6.42±0.18^{a}	6.90±0.03^{b}	7.11±0.16^{b}
6. Total bacteria count (log CFU/mL)	2.67±0.58^{b}	1.67±0.58^{ab}	1.34±0.58^{a}

Data are shown as the mean ± SD values .Means in a row superscripted with different lowercase letters are significantly different ($p < 0.05$).

A = November to December 2021, B =March to May 2022, C = July to August 2022

Table 3 Natural seawater properties collected from Rayong province at different time periods

Parameters	Collection period		
	A	B	C
1. Ambient temperature (ºC)	30.47±2.21[a]	35.37±4.18[a]	34.23±1.10[a]
2. Sea surface temperature (ºC)	30.10±0.12[a]	33.73±3.20[a]	33.83±0.76[a]
3. Salinity (ppt)	30.00±0.00[a]	29.33±0.58[a]	20.00±10.00[a]
4. pH	7.56±0.02[a]	8.08±0.04[b]	7.92±0.19[b]
5. Dissolved oxygen (mg/L)	6.76±0.06[a]	6.79±0.02[a]	7.24±0.17[b]
6. Total bacteria count (log CFU/mL)	1.33±0.58[a]	1.67±0.58[a]	1.33±0.58[a]

Data are shown as the mean ± SD values .Means in a row superscripted with different lowercase letters are significantly different ($p < 0.05$).

A = November to December 2021, B =March to May 2022, C = July to August 2022

Table 4 Natural seawater properties collected from Prachuap Khiri Khan province at different time periods

Parameters	Collection period		
	A	B	C
1. Ambient temperature (ºC)	28.20±0.72[a]	35.30±2.23[b]	34.70±0.89[b]
2. Sea surface temperature (ºC)	28.13±0.81[a]	31.00±1.00[b]	32.15±1.23[b]
3. Salinity (ppt)	30.00±0.00[a]	30.00±0.00[a]	31.00±0.00[a]
4. pH	7.89±0.01[b]	7.15±0.16[a]	7.87±0.13[b]
5. Dissolved oxygen (mg/L)	7.33±0.54[a]	7.54±0.07[a]	7.46±0.11[a]
6. Total bacteria count (log CFU/mL)	1.00±0.00[a]	2.67±0.58[b]	1.00±0.00[a]

Data are shown as the mean ± SD values .Means in a row superscripted with different lowercase letters are significantly different ($p < 0.05$).

A = November to December 2021, B =March to May 2022, C = July to August 2022

Table 5 Natural seawater properties collected from Chumphon province at different time periods

Parameters	Collection period		
	A	B	C
1. Ambient temperature (ºC)	25.23±0.57[a]	34.40±3.14[b]	29.43±2.78[a]
2. Sea surface temperature (ºC)	28.97±1.76[a]	31.33±1.53[a]	30.67±0.29[a]
3. Salinity (ppt)	29.67±0.58[a]	29.33±0.58[a]	30.67±1.15[a]
4. pH	8.07±0.02[c]	7.01±0.04[a]	7.76±0.01[b]
5. Dissolved oxygen (mg/L)	7.66±0.30 [a]	7.54±0.09[a]	7.61±0.14[a]
6. Total bacteria count (log CFU/mL)	1.33±0.58[a]	1.33±0.58[a]	1.67±0.58[a]

Data are shown as the mean ± SD values .Means in a row superscripted with different lowercase letters are significantly different ($p < 0.05$).

A = November to December 2021, B =March to May 2022, C = July to August 2022

Table 6 Natural seawater properties collected along the Gulf of Thailand coast for a year

Parameters	Provinces			
	CB	RY	PCH	CHP
1. Ambient temperature (ºC)	33.96±3.49[b]	33.36±3.29[ab]	32.73±3.63[ab]	29.69±4.50[a]
2. Sea surface temperature (ºC)	32.02±0.75[ab]	32.54±2.48[b]	30.43±2.00[ab]	30.32±1.58[a]
3. Salinity (ppt)	29.89±0.33[ab]	26.44±6.97[a]	30.33±0.50[b]	29.89±3.73[ab]
4. pH	7.72±0.37[a]	7.86±0.25[a]	7.64±0.38[a]	7.61±0.47[a]
5. Dissolved oxygen (mg/L)	6.81±0.33[a]	6.93±0.25[a]	7.44±0.29[b]	7.60±0.17[b]
6. Total bacteria count (log CFU/mL)	1.89±0.78[a]	1.44±0.53[a]	1.56±0.88[a]	1.44±0.53[a]

Data are shown as the mean ± SD values .Means in a row superscripted with different lowercase letters are significantly different ($p < 0.05$).

CB = Chonburi province, RY = Rayong province, PCH = Prachuap Khiri Khan province,

CHP = Chumphon province

Table 7 The biodegradation percentage of reference material at different time periods

Provinces	Biodegradation (%)		
	A	B	C
1. Chonburi	91.42±1.50[b]	82.16±9.64[ab]	76.66±3.30[a]
2. Rayong	90.74±3.35[a]	83.97±4.96[a]	83.22±5.13[a]
3. Prachuap Khiri Khan	82.53±5.27[a]	77.46±1.50[a]	78.77±4.29[a]
4. Chumphon	86.75±0.76[b]	76.94±5.36[a]	80.63±3.20[ab]

Data are shown as the mean ± SD values .Means in a row superscripted with different lowercase letters are significantly different ($p < 0.05$).

A = November to December 2021, B =March to May 2022, C = July to August 2022

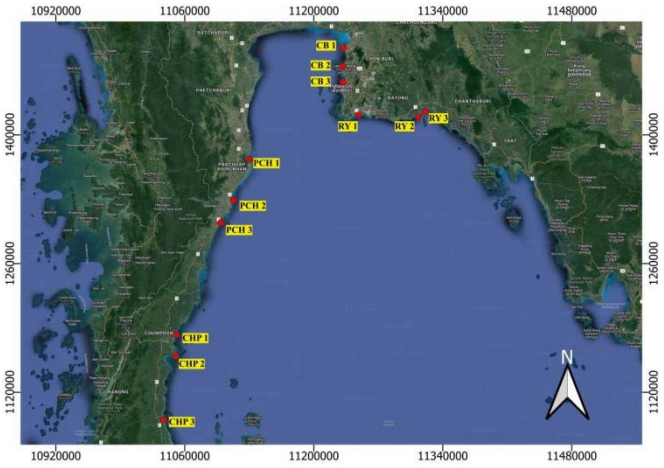

Fig. 1 Natural seawater collected areas along the Gulf of Thailand coast

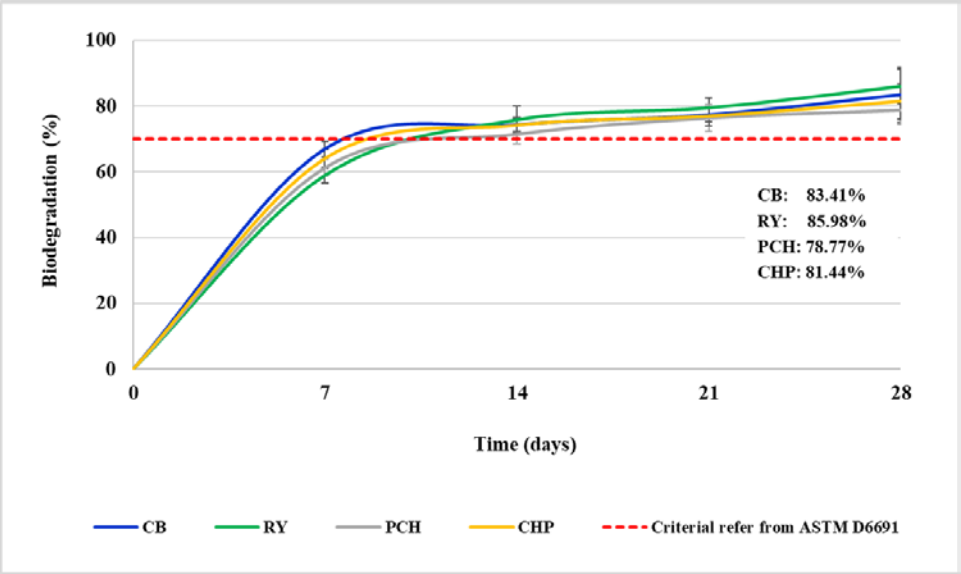

Fig. 2 The biodegradation percentage of reference material in collected natural seawater for a year, where data are mean ± SD values