Original paper

NATURAL BIODEGRADATION OF COPROSTANOL IN AN EXPERIMENTAL SYSTEM OF THREE ENVIRONMENTAL CONDITIONS OF JAKARTA WATERS, INDONESIA

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ABSTRACT

Constraint of using bio-indicator (coliform bacteria) as an indicator of domestic (sewage) pollution in the environment with high environmental stress encourages the discovering of other alternate indicators. Coprostanol has been proposed as a chemical indicator of domestic waste pollution, but most research on were conducted in the temperate (high latitude) region. The persistence of coprostanol in tropical region, especially in Indonesia, is still very poor. It is very important to understand the persistence of coprostanol in the nature, as one of the requirements to propose coprostanol as an alternate indicator of domestic waste pollution. In order to better understand the natural biodegradation of coprostanol, experimental system on three environmental conditions (river, river mouth, and coastal waters) was conducted. In April 2004, samples of water and surface bottom sediments were collected from each environmental condition in duplicate. Before the samples were put into aerated and non-aerated aquaria, about 35-40 g of surface bottom sediments were taken to analyze the initial concentration (C_0) of coprostanol. The sediments were subsequently sampled from each aquarium within a certain interval day to analyze the concentration of coprostanol (C_{10}, C_{20}, and C_{40}). The results showed that aeration plays not an important role in natural biodegradation of coprostanol. In average, the highest rate of coprostanol biodegradation is 0.438 µg/g day^{-1} in non aerated coastal water environment, where as the lowest was found in the non aerated river mouth environment (0.021 µg/g day^{-1}). Since coprostanol was degraded very slowly, and could be detected in the sediments of three environmental conditions, coprostanol has an excellent potency to be used as an alternate indicator of domestic wastes.

INTRODUCTION

In the last two decades, the reliability of coliform test as an adequate indicator of domestic (sewage) pollution, especially in urban coastal waters, has been questioned. Increasing intensity and variety of human activities, especially in urban regions, would increase the environmental stress in urban coastal waters. It is because wastes from terrestrial area were brought in from a

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variety of point sources, such as streams and drainage outfalls. Increasing volume of toxic and heated industrial wastes, the subsequent change of salinity from freshwater to seawaters, chlorination of wastewaters, and low dissolved oxygen were the constrains for the existence of coliform bacteria (Walker *et al.* 1982, Bartlett 1987, and Bachtiar 2002). Bartlett (1987) stated that the question of using coliform is mainly due to the lack of knowledge regarding die-off rates, particularly in saline waters. Manahan (1994) stated that die-off rates of bacteria in saline waters cauld be the result of the osmotic affect.

In order to better understand the sewage contamination in urban coastal waters an alternate indicator more persistence to the environmental stress is required. Various alternate indicators were proposed, and coprostanol showed a promised indicator of sewage contami-nation (Hatcher *et al.* 1977, Hatcher and McGillivary 1979, Brown and Wade 1984, Düreth *et al.* 1986, Holm and Windsor 1990, Coakley and Poulton 1991, Coakley *et al.* 1992, Bachtiar 1993, Bachtiar *et al.* 1996, Jeng and Hang 1994, Jeng *et al.* 1996, Chan *et al.* 1998, Bactiar 2002). Since most of these researches were conducted in temperate region, information related to coprostanol in tropical region, especially in Indonesia is urgently needed.

Several requirements for using a substance as an indicator are: a) related to the specific source, b) quantified (exist in field), and c) relatively conservative (the substance unchanged to become other substance or persistence). Coprostanol (5β-Cholestan-3β-ol) is the major fecal sterol of human, comprising 40 to 60 % of total neutral sterols excreted, and also detected in feces of mammals and chicken, but was not found in intestinal content of marine animals (Walker *et al.* 1982). Therefore it is a very specific indicator when coprostanol preset in marine environment

uniquely confirms recent or continuing sewage contamination.

In this work the persistence of coprostanol was studied using experimental system of three environmental conditions (river, river mouth, and coastal waters). The prime objective of this work is to evaluate the persistence of coprostanol as one of the requirements to use coprostanol as an alternate indicator of domestic waste in tropics, especially in Indonesia urban coastal waters.

MATERIALS AND METHODS

Water and Sediment Sampling

Duplicate of water and sediment samples were collected from three environmental conditions (river, river mouth, and coastal waters) of Ciliwung River Jakarta in April 2004 (**Figure 1**). Five liters of water samples of each station were collected by using van Dron water sampler about 10 cm of the upper most. These samples were immediately put into dark bottles and stored in a cool box (about 5°C) during fieldwork. About 5 kg of sediment samples of each station were collected using van Veen grab sampler. The grab sampler was carefully used to ensure that the surface of sediment was as undisturbed as possible.

Field Measurements

Water Quality Checker was used to measure on site temperature, conductivity, dissolved oxygen, and pH. Refractometer was used for salinity, and Secchi disc for water clarity.

Experimental System

Duplicate sediment and water samples from each station were put into duplicate aerated and non-aerated aquaria. The

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sediment samples of each station were homogenized by mixing them manually before put into the aquarium. The aeration system was installed only to aerate the water column and undisturb the surface of sediments. About 35-40 g of sediments was collected from each sample for analyzing the initial concentration of coprostanol (C_0). The sediments were taken as samples from each aquarium with certain days period to understand the change of coprostanol concentration (C_{10}, C_{20}, and C_{40}).
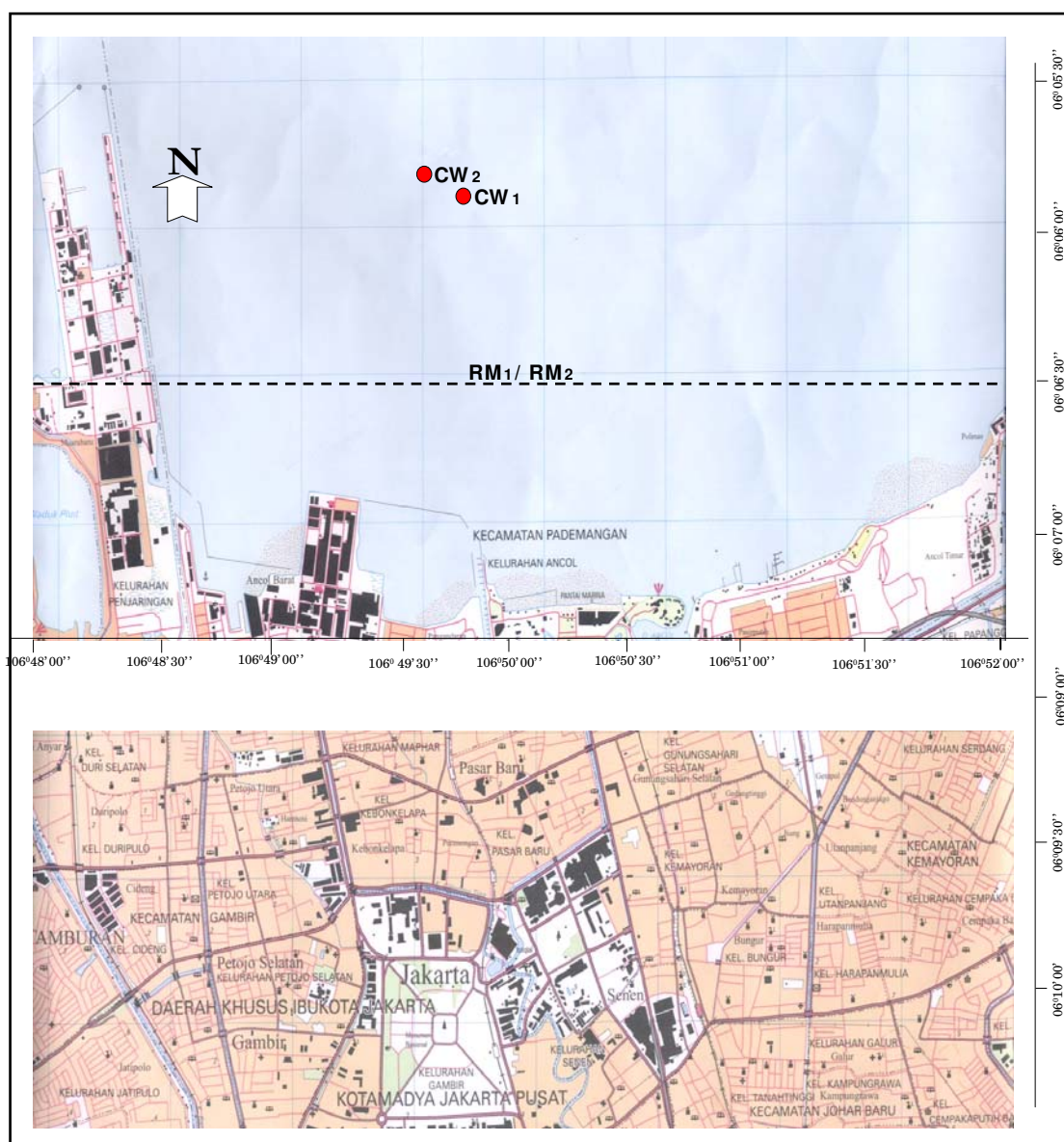

Fig. 1 Sampling sites for water and sediments of three environmental conditions: river (R), river mouth (RM), and coastal waters (CW) of Ciliwung River, Jakarta.

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Coprostanol Analysis

The concentration of coprostanol in sediment samples was analyzed using UV Spectrophotometer which applies to the standard curve of coprostanol. The analytical procedure is as follows:

Standard curve of coprostanol in ethanol

Preliminary UV spectrophotometer analysis of coprostanol standard in ethanol was conducted to find the optimum wave length. The result showed that the optimum wave length for coprostanol in ethanol was 250 nm. Based on that result, five concentration of coprostanol standard: 5, 10, 15, 20, and 25 ppm in ethanol were analyzed using UV Spectrophotometer with λ 250 nm to understand the correlation of absorbance and concentration of coprostanol standard called the standard curve of coprostanol in ethanol.

Sample preparation

- About 35-40 g of sediment samples were twice extracted using diethyl ether for 6 hours. The extract was collected and the sediments were allowed to dry by placing them in the oven (60°C) for a few days. The weights were continuously recorded until their value no longer changed.
- The extracts were evaporated in room temperature to near dryness by stirring using orbital shaker. Ethanol (2 ml) was used to dilute the extract.
- Total organic content (TOC) and grain size of sediments were analyzed to support interpretation of the data.

UV Spectrophotometer

Each extract in ethanol of sediment samples was analyzed using UV Spectrophotometer with λ 250 nm to know the absorbance. Based on the absorbance values of each extract, concentration of coprostanol in ethanol could be determined by using the standard curve of coprostanol in ethanol. The concentration of coprostanol in sediment samples was calculated based on the concentration of coprostanol in ethanol and the dry weight of sediment residue of each sample.

Biodegradation Rate

Biodegradation rate of coprostanol in each environmental condition was determined by using the formula as follows (Bartlett, 1987):

$$R_s = \frac{C_0 - C_t}{\Delta t}$$

(1)

where R_s = biodegradation rate of coprostanol

C_0 = initial concentration of coprostanol

C_t = concentration of coprostanol at t days

Δt = duration of experiment (days)

RESULTS AND DISCUSSION

Results

The results of coprostanol analyses were listed in **Table 1** and showed in **Figure 2**. Based on the results of coprostanol analyses, the rate of coprostanol biodegradation is determined, and listed in **Table 2** and showed in **Figure 3**. The results of TOC and grain size analysis were listed in the **Table 3**.

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Table 1 .Coprostanol analysis in natural biodegradation experiment (μg/g)

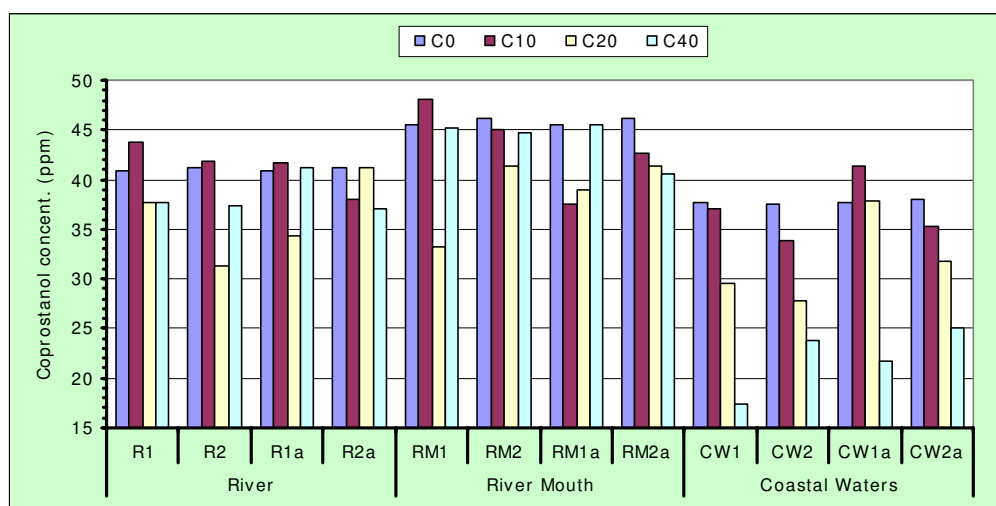| Environmental condition	Co	C_{10}	C_{20}	C_{40}
River				
R1	41.24	43.77	37.66	37.72
R2	41.24	41.78	31.25	37.35
R1a	41.24	41.68	34.41	40.90
R2a	41.24	38.05	41.22	37.13
River Mouth				
RM1	45.46	48.04	33.22	45.28
RM2	46.19	45.02	41.44	44.66
RM1a	45.46	37.53	38.91	45.45
RM2a	46.19	42.63	41.31	40.60
Coastal Waters				
CW1	37.64	36.99	29.53	17.45
CW2	38.05	33.82	27.78	23.26
CW1a	37.64	41.33	37.86	21.66
CW2a	38.05	35.32	31.71	25.11

Fig. 2 The results of coprostanol analysis in sediment of three environmental conditions (river, river mouth, and coastal waters) of Ciliwung river, Jakarta.

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Table 2 The rates of natural biodegradation of coprostanol (μg/g day^{-1}) in three environmental conditions of Ciliwung river, Jakarta

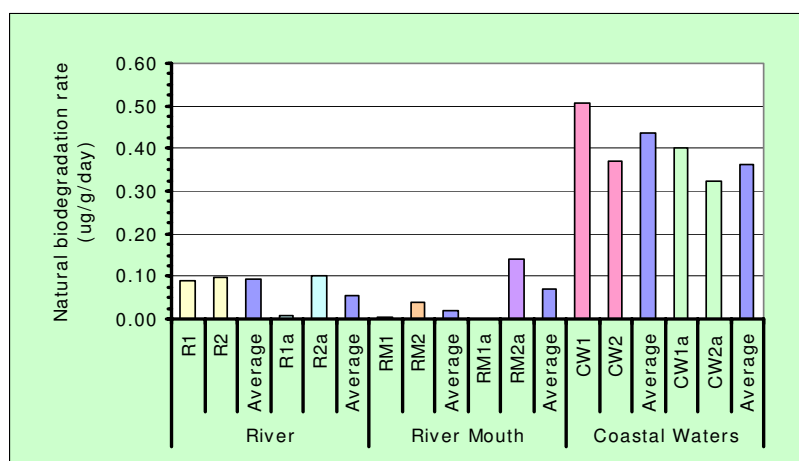| Environmental Condition	Non-aerated				Aerated			
	I	**II**	**Average**	**SD**	**I**	**II**	**Average**	**SD**
River	0.088	0.097	0.093	0.006	0.009	0.103	0.056	0.066
River Mouth	0.004	0.038	0.021	0.024	0.0002	0.140	0.070	0.099
Coastal Waters	0.505	0.370	0.438	0.095	0.400	0.324	0.362	0.054

Fig. 3 The rates of natural biodegradation of coprostanol in three environmental conditions (river, river mouth, and coastal waters) of Ciliwung River, Jakarta (April 2004).

Table 3 TOC and sediment grain size analysis

Sample ID	TOC (%)	Percentage of Grain Size			Grain Size
		Sand	**Silt**	**Clay**	
R1	37.80	9.83	22.65	67.52	Silty clay
R2	36.23	11.01	20.12	68.87	Silty clay
RM1	40.68	10.42	19.57	70.01	Silty clay
RM2	42.21	11.03	20.55	68.42	Silty clay
CW1	31.93	11.95	22.40	65.65	Silty clay
CW2	29.97	12.14	23.36	64.50	Silty clay

Discussion

The results of coprostanol analyses (**Table 1** and **Figure 2**) showed the fluctuation of coprostanol concentration. The concentration of coprostanol did not always show a gradual degradation. This is a common occurrence difficult to be eliminated in a natural biodegradation experiment (Bartlett, 1987), where the sediments are not well homogenized. Because of that, it could not be assured that their

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characteristic (grain size), coprostanol, and microorganism in the sediment are well distributed. Beside that, random sediment sampling in the aquaria is another factor that affects the fluctuation values of coprostanol concentration. As a result, the concentration of coprostanol does not decrease gradually, and the natural biodegradation rates of coprostanol also vary depending on the values of C_0 and C_{40}.

(**Table 2** and **Figure 3**) showed that the highest average of coprostanol natural biodegradation rate (0.438 µg/g day^{-1}) occurred in the sediment non-aerated coastal water environment where the initial concentration of coprostanol is relatively low (37.64 – 38.05 µg/g) compared to the initial concentrations of coprostanol in the river sediments (41.24 µg/g) and river mouth sediments (45.46 – 46.19 µg/g). The lowest rate of natural coprostanol biodegradation occurred in sediments of non-aerated river mouth environment (0.021 µg/g day^{-1}). The results show that aeration which only aerated water column does not play important role in biodegradation of coprostanol in sediment. These phenomena indicate that to determine the natural biodegradation rate many factors need to be considered.

The initial values of coprostanol concentration (C_0) should have very important role in determining the natural biodegradation rate of coprostanol. However, the results indicated that not always high initial concentration of coprostanol will have high natural biodegradation rates.

The lowest average natural biodegradation rate (0.021 µg/g) was found in the sediment of river mouth with non-aeration treatment, where the sediments contained a relatively high concentration of coprostanol (45.46 – 46.19 µg/g) and organic material (40.68 – 42.21 %). Based on the data, it is clear that homogenety of the sample has important role in determining the natural biodegradation rate. This also indicates that coprostanol degraded relatively very slowly. As a result coprostanol would be accumulated in the sediments and could be used as an alternate indicator of domestic waste pollution in urban coastal waters.

CONCLUSSION

To better understand the persistence of coprostanol as one of the requirements to be used as an indicator of domestic waste, it is necessary to conduct such as a natural biodegradation studies of coprostanol in other cities in Indonesia representing different environmental condition to the condition of Jakarta. This is because different environmental conditions would suppose to have a certain characteristic of coprostanol natural biodegradation rates.

ACKNOWLEDGEMENTS

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