DEGRADATION OF NATURAL HORMONE IN ANAEROBIC COMPLETELY STIRRED TANK REACTOR

(*DEGRADASI NATURAL HORMON DENGAN MENGGUNAKAN ANAEROBIC COMPLETELY STIRRED TANK REACTOR*)

Rahyani Ermawati
Balai Besar Kimia dan Kemasan, Jakarta

ABSTRACT

Expand and growing industrial activities have increased the possibility to release the chemicals, called Endocrine Disrupting Chemicals (EDCs), into the environment. In this study, an efficient treatment process for removal of natural hormone especially for degradation of natural steroid hormone (17 ß-estradiol or E2) was develop. A mesophilic anaerobic completely stirred tank reactor fed with artificial wastewater was constructed to study the anaerobic 17 ß-estradiol degrading mechanism. From the result of degradation, more than 95% of 17 ß-estradiol was successfully degraded and about 60% of them were converted to estrone (E1) which shows lower toxicity than 17 ß-estradiol (E2). The result of this study indicated that anaerobic treatment was verified to be an alternative effective method to remove the natural steroid hormone.

Key words: *Artificial wastewater, natural steroid hormones, mesophilic anaerobic Completely Stirred Tank Reactor (CSTR).*

ABSTRAK

Perkembangan dan pertumbuhan industri akan meningkatkan bahan-bahan kimia dari proses industri yang terbuang ke lingkungan. Bahan-bahan kimia ini lebih dikenal dengan nama Endocrine Disrupting Chemicals (EDCs). Pada penelitian ini telah dilakukan pengolahan untuk menghilangkan natural hormon khususnya natural steroid hormon. Mesophilic anaerobik completely stirred tank reactor (anaerobic CSTR) telah didisain dengan menggunakan limbah buatan untuk mempelajari mekanisme degradasi dari 17 ß-estradiol. Hasil penelitian ini menunjukkan bahwa lebih dari 95% 17 ß-estradiol berhasil diuraikan dan 60% dari peruraian tersebut berubah menjadi estron (E1), dimana estron toxicity lebih rendah jika dibandingkan dengan 17 ß-estradiol. Dari penelitian ini dapat disimpulkan bahwa anaerobic treatment dapat dijadikan alternatif yang efektif untuk menghilangkan natural steroid hormon yang ada dilingkungan.

Kata kunci: *Limbah buatan, natural steroid hormone, mesophilic anaerobic Completely Stirred Tank Reactor (CSTR).*

INTRODUCTION

In recent years, there has been considerable growing in scientific concerns about the potential adverse effects of the chemicals that have potential to alter the normal function of the endocrine system in human and wildlife. Expand and growing industrial activities have increased the possibility to release these chemicals, Endocrine Disrupting Chemicals (EDCs), into the environment. EDCs have a potential to mimic natural hormones by binding the hormone receptors therefore causing effects

on growth, behavior, reproductive and immune functions of human and wildlife. EDCs can disturb the endocrine system of many species even in a very low concentration. Since 1996, the US Environmental Protection Agency (US EPA) Office of Research and Development has considered the endocrine disruption as one of the top six research priorities.

Among EDCs, the natural steroid hormones released by human and livestock showed big effect on the wildlife in surface water for their relatively higher activities. Therefore, the detection of these natural hormones in the water environment, the effective wastes/wastewater treatment techniques designed to remove these natural hormones, are of great importance.

Natural estrogens hormones (E2, E1 and E3) in the environment were excreted from human or farm animals and induce of the biological effects at low concentration. Among of the natural estrogens hormones, E2 is the most potent and have been suggested as one of the major groups of substances which cause endocrine disruption effect on human and wildlife (Lee *et al.*, 2002). E2 was detected in many water systems (Isii *et al.*, 2002), effluents from sewage treatment plant (STP) (Fujii *et al.*, 2002) and agriculture industries (Raman *et al.*, 2004). The degradation of natural estrogenic hormones under the aerobic conditions was well studied (Ying *et al.*, 2003; Ternes *et al.*, 1999), and it showed that they could be easily removed from the wastewater when using activated sludge method. Meanwhile, the researches on the degradation of these natural steroid hormones under anaerobic conditions were limited and in general it was considered that anaerobic degradation of these natural steroid hormones is much more difficult compared to that in aerobic conditions (Ying *et al.*, 2003).

On the other hand, the results Ermawati *et al.*, 2007 shows that most of estrogenic activity in cow manure waste was removed under anaerobic treatment process suggested that anaerobic treatment should be an alternative useful method for the removal of these hormones especially when treating wastes with high concentration of organic materials. However, how these natural hormones were degraded under anaerobic condition is still unknown. Therefore, in this section, a simple anaerobic methane fermentation system fed with artificial wastewater containing 17 β-estradiol (E2) was constructed. And then the degradation efficiency and the fate of 17 β-estradiol (E2) were investigated.

MATERIALS AND METHODS

A. Artificial Wastewater

Artificial wastewater contain of carbon and energy source with added of trace element solution were used in this research as supplying for microorganism growth. Artificial wastewater with butyrate and 17β-estradiol as carbon and energy source was prepared as follows (g/L): 17β-estradiol (Wako Pure Chemical Industries , Osaka, Japan), 100μg (C_4); $CH_3CH_2CH_2COOH$, 11.7; $CH_3CH_2CH_2COONa$, 3.67; KH_2PO_4, 0.3; $KHCO_3$, 4.0; NH_4Cl, 1.0; NaCl, 0.6; $MgCl_2.6H_2O$, 0.82, $CaCl_2.2H_2O$, 0.08; L-Cystein-HCl-H_2O, 0.1 g; 10 ml of the DSMZ medium 318 trace element solution (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany) containing 52.6 μg/l of Ni^{2+} and 61.2 μg/l of Co^{2+}, and 10 ml of the DSMZ medium 318 vitamin solution without B_{12}. The total organic carbon (TOC) concentration of the artificial wastewater was approximately 8,000 mg/l.

B. Construction and Operation of the Meshophilic Anaerobic Completely Stirred Tank Reactor (CSTR)

The anaerobic mesophilic chemostat was operated using completely stirred tank reactor with a working volume 1.8 L (V) and was mixed using a magnetic stirrer. It was expected that using this reactor all the wastewater will completely mixed and the reactor easy to maintain during operation period. The other advantage of this reactor was produced the methane gas as energy source. The temperature was maintained at 37°C by immersing the reactor in a thermostated water-bath and pH was maintained automatically at 7.0 by feeding 1 N HCl solution through a pump which was controlled by a pH controller. 1.2-L portion of mesophilic digested sludge from the Kumamoto-Hokobu sewage treatment plant (Kumamoto City) was washed twice with a artificial waste without 17β-estradiol under

anaerobic conditions, was diluted to 1.8 L, and was then placed in CSTR. Artificial waste water containing butyrate as the sole carbon source was fed into the CSTR at a dilution rate of 0.05 d^{-1} (flow rate was 0.09 L/d). Biogas was collected in a gasholder. After 38 days continuous operation, artificial wastewater containing 17 β-estradiol were fed into reactor. Figure 1 shows the schematic of the CSTR.

C. Sample Concentration

The concentration of sample using the method modified from the procedure described by previous studies (Raman *et al.*, 2004), were carried out as follows: The samples were extracted in triplicate. Sample from reactor about 20 mL was placed in a 50 mL glass vial with 10 mL of ethyl ether, and were shaken automatically for 30 min at on a vertical shaker and then centrifuged at 2300 x *g* for 10 min, and the supernatant was collected. Ethyl ether being extracted from each sample was collected followed by evaporation using centrifugal concentrator to dry it. The residue was dissolved in DMSO and then used as a test sample in the yeast two-hybrid assay and ELISA measurement. In case sludge and supernatant samples, a 20 mL sample was centrifuge at 7000 rpm for 10 min, then the supernatant was collected and sludge sample was added with 20 mL of deionized water and continuing as procedure for extracted as above.

D. Measurements of 17 β-estradiol (E2) and Estron (E1)

The concentration of E1 and 17β-estradiol (E2) was determined by using ELISA (Assay Design, Inc., Ann Arbor MI) according to the manufacturer's instructions. The ELISA method was selected for measurement of E1 and E2 in the influent and effluent according to the sensitivity of this method without time consuming. The recovery efficiencies of E1 and E2 by the ELISA measurement were 110% and 112%, respectively.

E. Other Analytical Methods

All parameters of the influent and the effluent were measured regularly three times a week. Total organic carbon (TOC) and volatile fatty acids (VFA) were measure after centrifugation at 8000 x *g* for 10 min. TOC was analyzed by using a TOC auto analyzer (TOC-500; Shimadzu, Kyoto Japan) according to standard methods (Namiki, 1986). VFA was analyzed by a post-label method using a HPLC as described in our previous paper (Kida *et al.*, 1993). Total solid (TS), total volatile solid (TVS), BOD_5, suspended solid

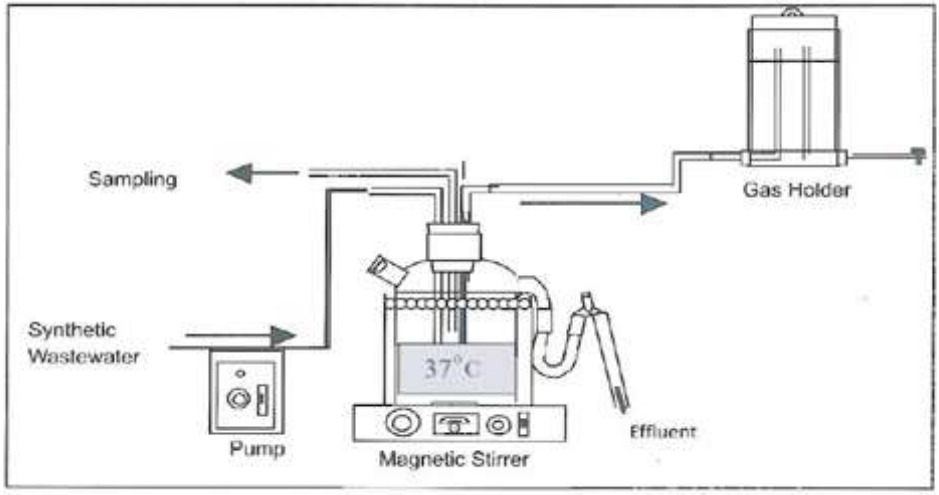

Figure 1. Schematic of the mesophilic anaerobic completely stirred tank reactor

(SS) and volatile suspended solid (VSS) were analyzed in according to standard methods (Namiki, 1986). Color index was measured by spectrophotometric method. CODcr was measured using the HACH method (method 8000, HACH Company). The methane content of the biogas was measured using a gas chromatograph equipped with a thermal conductivity detector (TCD) (KOR-2G, GL Science Co., Tokyo) with a packed column (Porapack Q, GL Science Co., Tokyo).

RESULT AND DISCUSSION

A. Operation Result of the Mesophilic Anaerobic CSTR

Artificial wastewater containing of butyrate as the sole carbon source was fed into the completely stirred tank reactor (CSTR) at a dilution rate of 0.05 d^{-1}. After 38 days operated the 17β-estradiol were added in the artificial wastewater at a concentration of 100 µg/L. After 50 days operation at a dilution rate of 0.05 d^{-1}, MLVSS concentration were reached stable, being approximately of 1 g/L. Average biogas production rate was 650 mL/L/day. Methane and CO_2 concentration in the biogas were approximately 70 % and 20 %, respectively. TOC removal efficiency was more than 99 % and VFA was lower than 100 mg/L (Figure 2).

B. Degradation and Behavior of 17β-estradiol (E2) in the Mesophilic Anaerobic CSTR Measured by ELISA

Using ELISA method, the concentration of E1 and E2 in the culture broth in the reactor were determined approximately once a weak (Figure 3). The theoretical accumulation of E2 in the reactor under the condition of no E2 degradation occurred was shown in the same figure. The concentration of E2 in the reactor was lower than 5 µg/L. As the concentration of E2 in the influent was 100 µg/L, the degradation efficiency of E2 kept at 95% during whole operation period of about 5 month. However, even no E1 was fed into the reactor, E1 was detected continuously in the reactor. At the time the feeding of E2 was started, the increase of E1 also started and it reached a highest concentration of about 78 µg/L after 75 days operation. After that, some of E1 accumulated was degraded and E1 then kept at approximately 55 µg/L over 3 month's operation.

The results above suggested that about 60% of E2 degraded was converted to E1 and 40% of E2 removed was converted to other intermediates or completely degraded in the

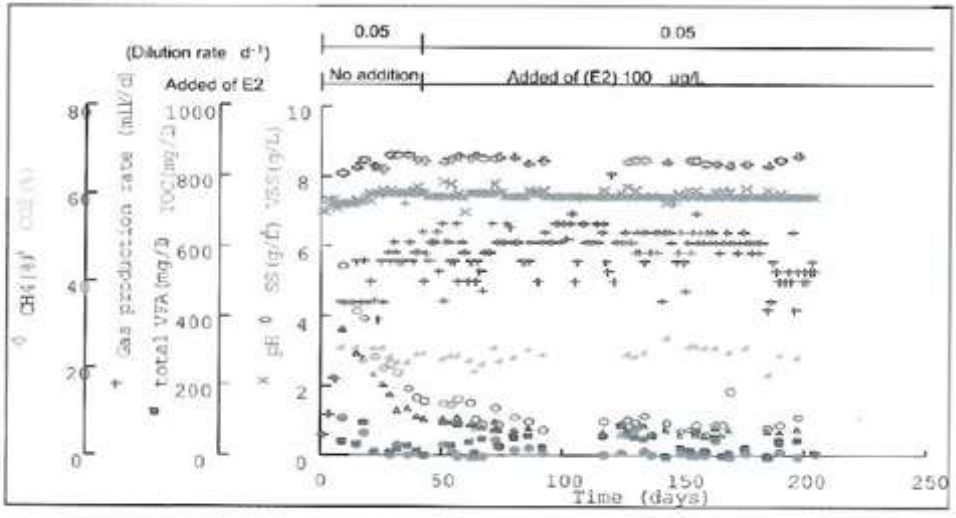

Figure 2. Time course of the performance of the mesophilic anaerobic CSTR

anaerobic reactor. The result that the concentration of E1 kept at the high level in the reactor indicated that degradation of E1 under the anaerobic condition was relatively difficult compared to that of E2. Similar result was also reported by another research group (Cynthia et al. 2006). However, different result was reported by Joss *et al.*, (2004). In their study, the reduction of E1 to E2 occurred under the anaerobic conditions, which had not taken place in this study for the big difference between the concentration of E1 and E2 in the reactor. Without the information of intermediate products, it was difficult to deduce the metabolic pathway of E2 under anaerobic condition from these results now obtained. Many researchers reported that under the aerobic condition, E2 was first oxidized to E1 and E1 was further degraded completely. However, how E2 was degraded under anaerobic condition had not reached a final conclusion.

C. Implementation of this Results to the Industrial Effluents

- The implementation of these results to the industrial effluents should be applied in the covered of hazardous wastewater treatment. Moreover, the final effluent should be save to the environment.
- Indonesian National Standard for the wastewater treatment effluent should be more covered of toxic compounds.
- Wastewater testing laboratory in Indonesia should be strengthened by capability of human's resources for the analyzing of toxic compounds.
- Standard method of toxic compounds could be socialized to the industries and testing laboratories as main holders.

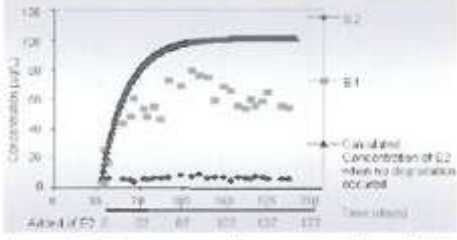

Figure 3 . Time course of the concentration of E2 and E1 (ELISA) in mesophilic anaerobic completely stirred tank reactor (CSTR)

CONCLUSIONS

Anaerobic 17 β-estradiol (E2) degrading chemostat was successfully constructed and the E2 characteristic degradation was studied. 95% of E2 fed into the reactor was degraded. 60% of E2 degraded had been converted to E1. It can be concluded that it is possible to use an anaerobic treatment method to degradation of toxic compounds such as E2. Though E2 was not completely degraded to mineralizes as CO_2 and H_2O in the final results in our study.

To clear the E2 anaerobic degradation pathway, further studies are needed. The analysis of intermediates is one important part, which can provide basic information for proposing possible pathways. The study of microorganisms related to the degradation of E2 is another necessary part. Identification of related microorganism from the complex microbial community is of course very difficult and the analysis method combining the molecular biological techniques and isotope probing technique might provide perspective results. The genome analysis of the related microorganisms therefore can give us intact information of the E2 degradation pathway.

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