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Anaerobic Digestion of MSW Spiked with BPA

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Abstract: This study aims to investigate the effects of bisphenol A (BPA) on the MSW (municipal solid waste) biodegradation in anaerobic bioreactors. BPA 10 and 100 mg L^{-1} daily fed bioreactors were found to stimulate the biogas production rate after about 126 days' operation while that of BPA 500 mg L^{-1} daily fed ones was inhibited gradually from the beginning. Enhancement or inhibition of MSW biodegradation was thought to be the resulting exposed BPA soluble levels of ~10 - ~13, ~11 - ~30 and ~30 - ~102 mg $L^{-1}$for 10, 100 and 500 mg L^{-1} daily fed bioreactors compared to control (~4 - ~10 mg L^{-1}). PCR (polymerase chain reaction) and DGGE (denaturing gradient gel electrophoresis) analysis also showed that *Methanosarcina* sp., *Methanosarcina barkeri* and *Methanomicrobiales archaeon* methane producing bacteria could adapt BPA levels (~10 - ~30 mg L^{-1}) at BPA 10 and 100 mg L^{-1} daily fed bioreactors resulting in the improvement of MSW anaerobic digestion and the enhancement of biogas production rates for about ~1 - ~3.5 times that of control between 126 and 305 days' operation.

Keywords: MSW; BPA; anaerobic digestion; PCR; DGGE.

1. Introduction

Municipal solid waste (MSW) containing bisphenol A (BPA) has been increasingly found in the environment due to the greater use of epoxy resin, polycarbonate and polyarylate plastic. BPA might cause the disorder of endocrine and intake of BPA higher than an intake level of 50 mg/kg body weight/day could lead to reproductive disability and genetic disease [1]. BPA in the environment and its toxicity study have been investigated in several reports [2-4]. BPA might be adsorbed onto the exposed

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environmental media resulting in various levels in the liquid and solid state [5]. Distribution of various levels might lead to the different impacts on the plant and animal metabolism resulting in a further risk on ecological environment and human health.

BPA was reported to be treated by physical and chemical process [6-10]. On the other hand, BPA can be treated with biological methods via microorganisms, enzymes, plants and mammals [11-15]**.** BPA treatments in literatures were mostly found in soil, wastewater and sludge. Few were reported in the biological treatment of BPA-containing MSW.

Anaerobic digestion performance of MSW, sludge etc. might be affected by several factors such as pH, temperature, organic matter content, carbon/nitrogen ratio, nutrients and particle size, solid retention time (SRT) and organic loading rate (OLR), pretreatment of acids, alkali, ultrasonication, radiation, microwave and ozonation, inoculums source, digester configuration, microbial community, co-digested materials, methane solubility and recalcitrant matters [16].

Currently, MSW is treated with physico-chemical and biological methods such as landfilling, composting, anaerobic digestion, incineration, gasification and resource recovery. Among them, anaerobic digestion is thought to be a potential methods for the MSW treatment due to the potential energy recovery of methane and hydrogen and digestate utilization for fertilizer and soil amendment. Compared to landfilling, composting and lincineration, MSW anaerobic digestion produces less leachate, potential toxic air pollutants and hazardous ashes contaminants. Thus, anaerobic digestion is still a promising option for MSW treatment. However, MSW has been found to be contaminated with many potential hazardous materials such as BPA, Nonylphenol (NP) and other food chemicals or additives which are discarded into the MSW stream after use. Performance of anaerobic digestion of MSW could be assessed by pH, chemical oxygen demand (COD), oxidation and reduction potential (ORP), electrical conductivity (EC), volatile solid (VS), volatile acids (VA), alkalinity, co-digested materials and microbial phase etc. Digestate evaluation for further utilization can be examined by toxicity characteristics leaching procedure (TCLP), Ames test, Microtox test and germination test etc [16]. Thus, anaerobic co-digestion of MSW with potential hazardous materials such as BPA, NA and others needs to be investigeted for deeper understanding of this potential beneficial treatment method.

MSW can be treated with thermal and biological methods that can recover the electricity or energy and resource utilization. As MSW is treated with biological methods such as landfilling, composting or anaerobic digestion, BPA could be detoxified by organisms' decomposition [15] of co-digestion of BPA and organic substrate such as MSW. MSW anaerobic digestion is a promising treatment method due to its potential methane, hydrogen and digestate utilization. Thus, this study aimed at investigating the effects of various BPA levels on the MSW anaerobic digestion that might provide the useful information for BPA containing MSW anaerobic digestion practice.

2. Materials and methods

2.1. Experimental materials

Experimental materials used in this study were MSW, BPA and anaerobic sludge seeding. BPA has a molecular weight of 228.29, its log K_{OW} is 3.32 and water solubility is 120 mg L^{-1}. BPA was purchased from Sigma-Aldrich.

MSW (6000 g dry weight) was comprised of office paper (30%), newspaper (30%), yard waste (35%) and food waste (5%)

blended with distilled water (94 L, 94000 g) to make the MSW TS ~6% (VS ~4%). This composition proportionally represents typical organic fractions of MSW (OFMSW) and could prevent the interference of unavoidable substances on the effects of anaerobic digestion of MSW spiked with BPA. Major elements of OFMSW such as C, H, O, N etc. were measured to be about 46%, 6%, 41% and 1.4%, respectively by elemental analyzer (Heraeus varioIII-NCH). The formula of MSW is calculated to be $C_{38.3}H_{60}O_{25.63}N$. Compared to Taichung city and Taiwan MSW, Taichung city and Taiwan MSW had the close C(%), N(%), S(%), Cl(%) values of about 20.55 and 17.38, 0.50 and 0.47, 0.47 and 0.5, 0.0625 and 0.07 respectively. C (46%) and N (1.4%) of synthetic MSW in this study showed higher compared to those of Taichung city and Taiwan MSW. However, C/N ratios of Taichung city, Taiwan and synthetic MSW were found to have close values in the order of 41.1, 39.68 and 32.86 suitable for anaerobic digestion. A C/N ratio ranging from 25 to 50 was reported to be suitable for the MSW composting and anaerobic digestion. Chemical compositions of lignin, α-cellulose, hemicellulose and ash content was thought to have similar ranges to that [17] as 17.7%, 47.4%, 6.9% and 12.3% (dry weight) respectively. Potential metal constituents of MSW were measured by induced couple plasma – optical emission spectroscopy (ICP-OES, IRIS Intrepid II, Thermal Electron Corporation) as reported by Lo et al. [18] and Lo and Liao [19]. Briefly speaking, ICP-OES was set at the required operational conditions. Incident energy was 1100W and reflective energy was <5 W. Observational mode of plasma was side on and the plasma height was 14 mm. Argon was used to produce the desired high temperature with RF power (1150 W). Nebulizer flow (25 PSI) and auxiliary flow were set at 0.75 and 0.5 L min^{-1}, respectively. Data acquisition was obtained with TEVA software (Thermo Elemental). All analytical methods followed the manual of manufacturer and the standard method for the examination of water and wastewater [20]. Metals levels in MSW compared to the results [21] seemed not to affect the anaerobic digestion.

Anaerobic sludge seeding (VS ~3%) was obtained from anaerobic digester of Fu-Tien municipal wastewater treatment plant in Taichung in central Taiwan. Matal levels in sludges were also measured by ICP-OES (IRIS Intrepid II, Thermal Electron Corporation) as reported by Lo et al. [18-19].

2.2. Anaerobic reactors operation

Start up of MSW biodegradation was by filling 2 liter anaerobic sludge seeding in anaerobic bioreactors (5 L) then conducted the bacteria acclimation by daily input of 100 mL MSW at 35℃ until total working volume was 4 L. Thereafter, 200 mL MSW output and input was operated at a SRT of 20 days. As the biogas production was monitored to be stable at gas collectors after about forty days, BPA levels of 0, 10, 100 and 500 mg L^{-1} (0, 2, 20 and 100 mg/200 mL MSW) blended with 200 mL MSW were put onto bioreactors and 200 mL digestates were taken out and filtered for parameters' analysis such as pH, COD, ORP, EC, alkalinity, VA, BPA, VS daily, weekly or monthly and microbial community was measured at day 150.

Basically, substrates (carbohydrate, fat, protein and lipid) such as MSW can be biodegraded to be biogas and other matters via hydrolysis, acidogenesis, acetogenesis and methanogenesis as shown in Figure 1. The formula of MSW is calculated to be $C_{38.3}H_{60}O_{25.63}N$ by elemental analysis. Thus, the theoretical biogas production (1.03124 L/g VS) such as methane, carbon dioxide and ammonia can be obtained ($C_{38.3}H_{60}O_{25.63}N + 9.735H_2O \rightarrow$

$19.8675CH_4 + 18.4325CO_2 + NH_3$). MSW biodegradation in batch mode follows the first order reaction [16, 18]. Assessment indicators of anaerobic digestion performances includes biogas production, pH, ORP, EC, COD, VS, VA, alkalinity, metals levels and microbial activity. pH between ~6.5 - ~7.5 is found to be suitable for anaerobic digestion. VA/alkalinity ratios less than 1 is thought to be more stable in the anaerobic digestion operation.

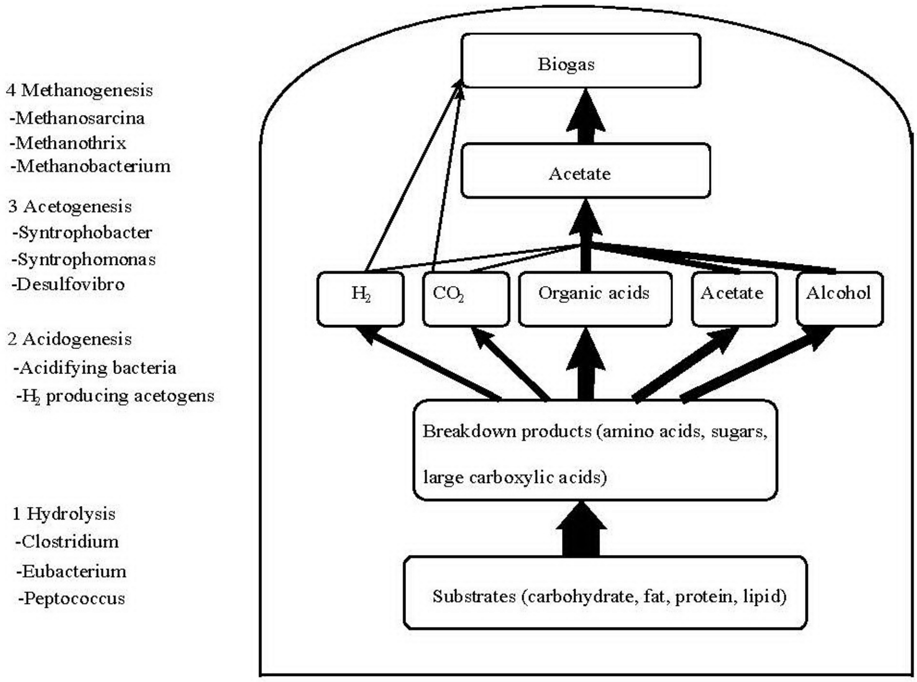


Figure 1. Proposed biochemical process for MSW anaerobic digestion

2.3. Chemical analysis

Biogas production was measured by biogas collectors with water replacement method daily. pH, COD, ORP, EC, alkalinity, volatile acids and VS were measured daily or weekly according to standard methods for the examination of water and wastewater [20]. pH, COD, ORP and EC were measured by pH 207 (Lutron), 765 Dosimat (COD titration meter), pH meter SP-2300 (SUNTEX) and Con 400 series (SUNTEX) respectively. Alkalinity and volatile acids were measured by titration methords. TS and VS were measured by 105℃ oven (DS45, Deng Yng) and 550℃ furnace (CMF 304, Cheng Jang).

BPA levels at anaerobic bioreactors were analyzed by high-performance liquid chromatography (HPLC, FLD, L-2485; Autosampler, L-2200; pump, L-2130, Hitachi) with a software of D-2000 HSM. The HPLC column was XTerra C18 (250*4.6 mm, 5μm). HPLC grade acetonitrile was purchased from Fisher.

Liquid BPA analysis was described briefly as follow [22, 23]: 1)filtering the MSW 10 mL from each bioreactor with 0.45 μm filter membrane and taking filtrate 5 mL into tubes, 2)then adding 2 mL CH_2Cl_2 into tubes, rotating the capped tubes in a shaker at 40 rpm for 10 min, 3)after standing still, then taking out the liquid for storage and adding another 2 mL CH_2Cl_2 into tubes for further extraction as step 2, 4)repeating the extraction as described in step 2 and 3 for two time, 5)collecting the storages and drying by evaporators for BPA residues. The BPA residue was dissoloved in 2 mL acetonitrile for HPLC analysis. The injection volume was 10μL. The mobile phase of water/acetonitrile was 55%/45% and flow rate was 0.8 mL min^{-1}. BPA fluorescence was monitored at an excitation of 225 nm.

2.4. Microbial analysis

Microbial structure analysis (bacteria and archaea) was according to the previoue reports [24-26]. Samples 400 mL was collected at day 150 from each control, 10, 100 and 500 mg L^{-1} BPA fed bioreactors as well as the anaerobic sludge seeding 400 mL from the anaerobic digester from Fu-Tien municipal wastewater treatment plant. Each total 400 mL samples were assumed to have 10 g higher and pretreated with centrifugration for DNA extraction by UltraCleanTM Soil DNA Isolation Kit (Mo Bio) for further polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE) analysis [24-26].

DNA sequence was analyzed by Mission Biotech (Taiwan). The results were compared by Nucleotide Blast in the National Center for Biotechnology Information (NCBI) network. Phylogenetic tree was drawn by MEGA 4 software after sequence comparison.

3. Results and discussion

3.1. Biogas production

Initial biogas production rate at 10, 100 and 500 mg L^{-1} BPA fed bioreactors decreased quickly compared to the control. The microorganisms at the 10 and 100 mg L^{-1} BPA fed bioreactors acclimated and adapted to the BPA levels similar to the evolutionary phenomenon reported by Bell and Gonzalez [27] while those at the 500 mg L^{-1} BPA fed bioreactors failed to tolerate the BPA concentration after 30 days. After acclimatization and adaptation for 126 days, the biogas production rate at 10 and 100 mg L^{-1} BPA fed bioreactors reached highest to about ~1 - ~ 3.5 times that of the control between 126 and 305 days' operation (Figure 2).

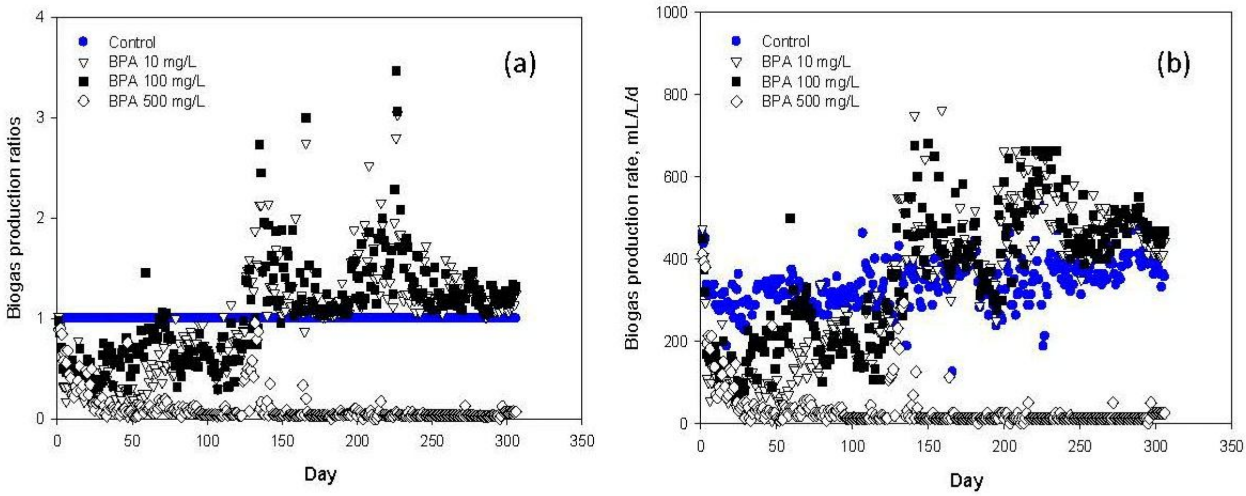


Figure 2. Biogas production ratios (a) and biogas production rates (b) in various BPA dosed and control reactors

3.2. Chemical parameters

Chemical measurements of anaerobic parameters can be found in Figure 3. Most pHs in four bioreactors were similar and were found to be about ~6.2 - ~6.8. ORPs also had similar values and were measured to range between ~ -262 and ~ -70 mV. ECs were mostly found to be ~2.7 - ~4.6 ms cm-1 and most alkalinity were between ~32 and ~285 mg L-1. VAs were mostly found in the range of ~20 - ~1120 mg L-1. CODs in four bioreactors were measured to be about ~2520 - ~41920 mg L-1. VAs and CODs in conrol bioreactors were found comparatively lower than those in 10, 100, and 500 mg L-1 BPA fed bioreactors. Higher CODs in higher BPA fed bioreactors came mainly from the BPA accumulation. These anaerobic parameters appeared to be suitable for MSW anaerobic digestion.

BPA might be biodegraded into intermediates [9-10, 13] by microorganisms or chemical oxidants and might be adsorbed onto MSW and sludge admixture leading to the soluble levels as can be found in Figure 4. BPA levels at control, 10, 100 and 500 mg L^{-1} BPA fed bioreactors were analyzed to be ~4 - ~10, ~10 - ~13, ~11 - ~30 and ~30 - ~102 mg L^{-1} over 305 days' operation respectively. BPA levels at the 10 and 100 mg L^{-1} BPA daily fed bioreactors had the potential for microorganisms to adapat and increase the biogas production after 126 days' operation. However, it was inhibited completely with the 500 mg L^{-1} BPA daily addition after 30 days. Metal analysis was seen in Figure 5. Ca, Mg, K and Na (Figure 5(a)) was found higher than other trace metals (Figure 5(b)). All these metals levels compared to literatures [18, 21] had not the potential to stimulate the MSW anaerobic digestion.

3.3. Biological analysis

Results of DGGE analysis including bacteria and archaea. The archaea in the DGGE band (Figure 6) showed to contain *Methanosarcina* sp., *Methanosarcina barkeri* and *Methanomicrobiales archaeon* (methane producing) while bacteria were found to have *Sporobacterium olearium*, *Leuconostoc mesenteroides*, *Clostridiales* sp., *Sporobacterium olearium*, *Clostridium* sp., *Klebsiella pneumoniae* and *Spirochaetaceae* sp.. BPA was reported to be decomposed potentially by plants, microalgae, enzyme as well as microorganisms. Some investigations regarding the BPA removal by microorganisms were reported [11-15], however, few were related to the removal by the anaerobes. Yamanaka et al. [13] reported that *Sphingomonas* sp. strain BP-7 and *Sphingomonas* yanoikuyae BP-11R both could degrade 300 mg L^{-1} BPA efficiently in the presence of activated carbon (soluble leveles between ND and 3.1 mg L^{-1}) without releasing 4-hydroxyacetophenone intermediate from BPA into the medium. This BPA soluble levels was closed to that of 10 and 100 mg L^{-1} BPA fed bioreactors in this study. It is noted that a critical degradation solubles levels between 100 and 500 mg L^{-1} (soluble levels beween ~30 and ~102 mg L^{-1}) might need further investigation.

4. Conclusions

Biogas production rate at 10 and 100 mg L^{-1} BPA daily fed MSW anaerobic bioreactors (BPA soluble levels ~10 - ~30 mg L^{-1}) was enhanced ~1 - ~3.5 times that of control after 126 days over all 305 days' operation period while that at 500 mg L^{-1} BPA daily fed ones (BPA soluble levels ~30 - ~102 mg L^{-1}) were gradually inhibited compared to that of control.

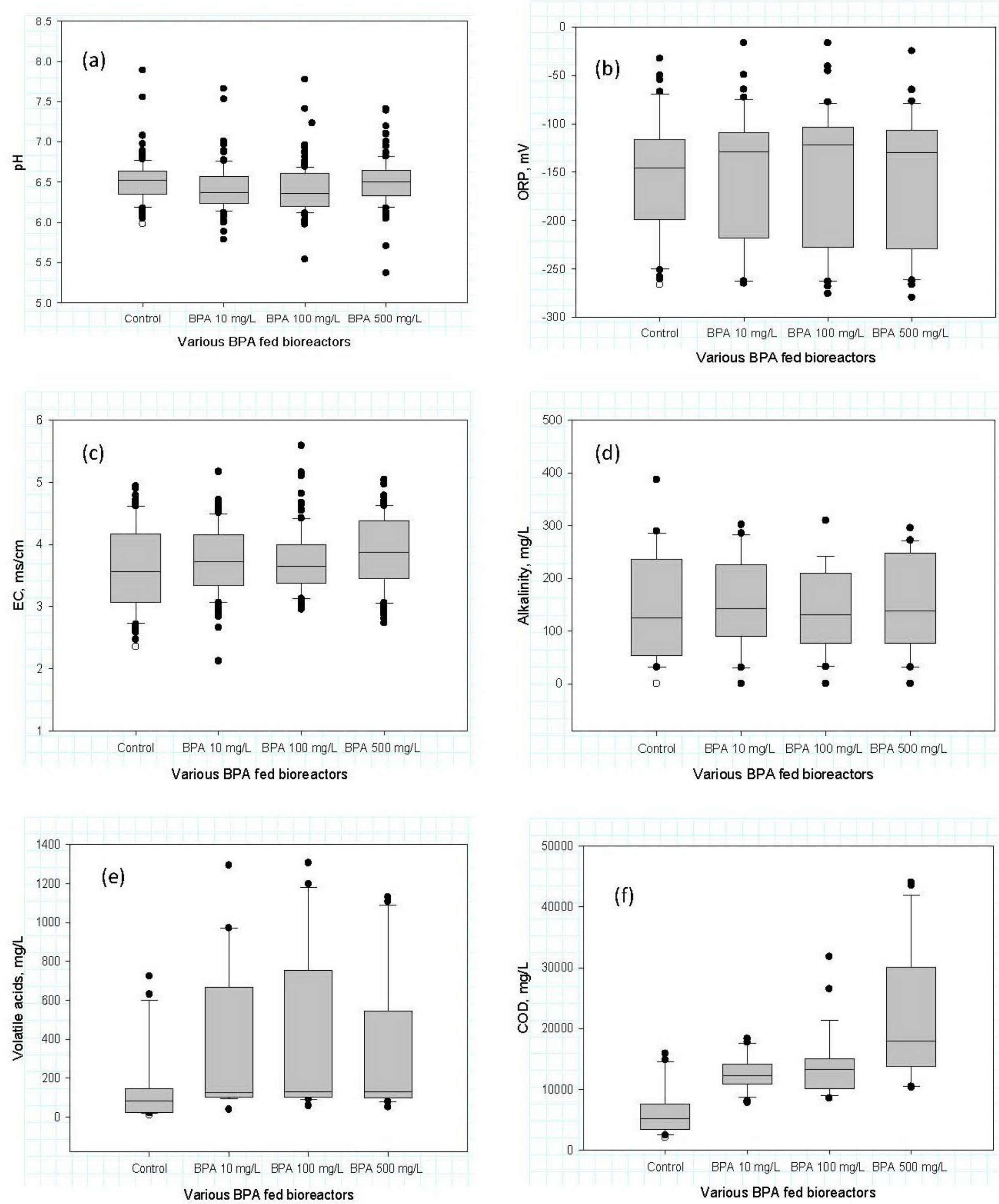


Figure 3. pH (a), ORP (b), EC (c), alkalinity (d), VAs (e), and COD (f) in the control, 10, 100 and 500 mg L^{-1} fed anaerobic bioreactors

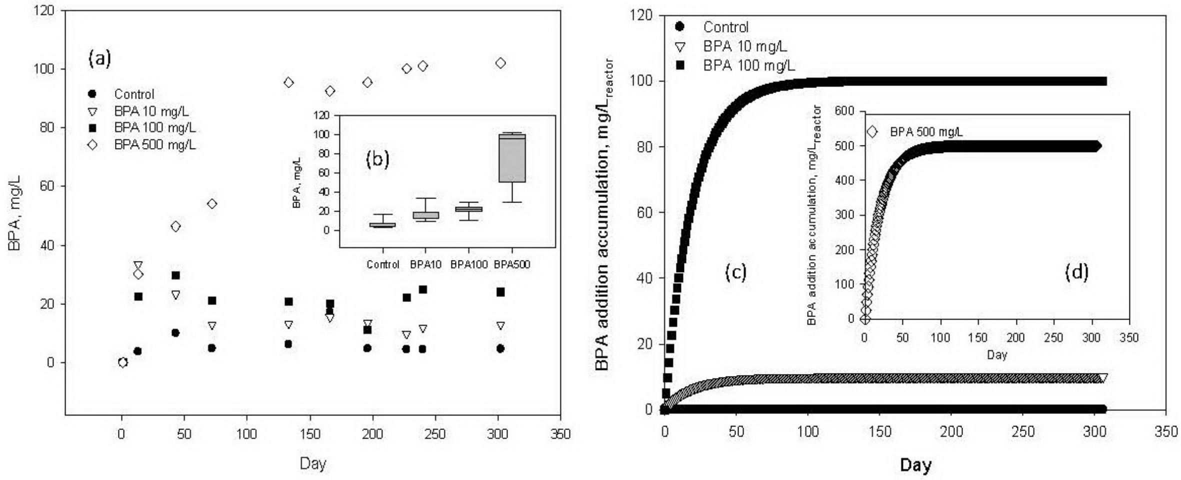
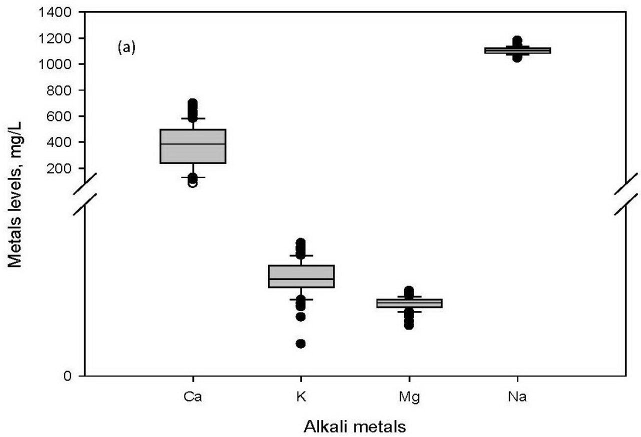


Figure 4. BPA levels (a and b) and BPA addition accumulation (c and d) at various BPA dosed and control reactors



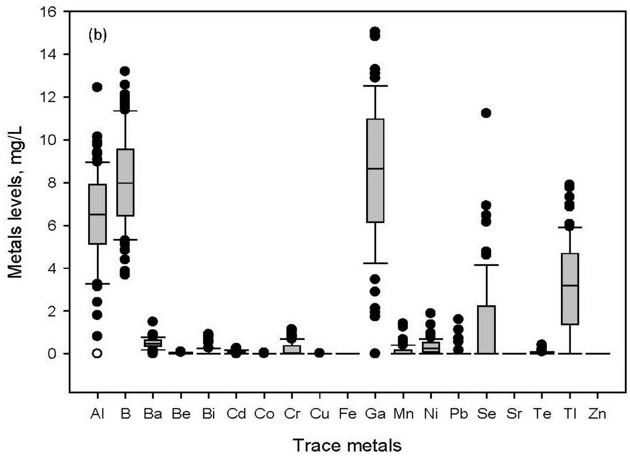
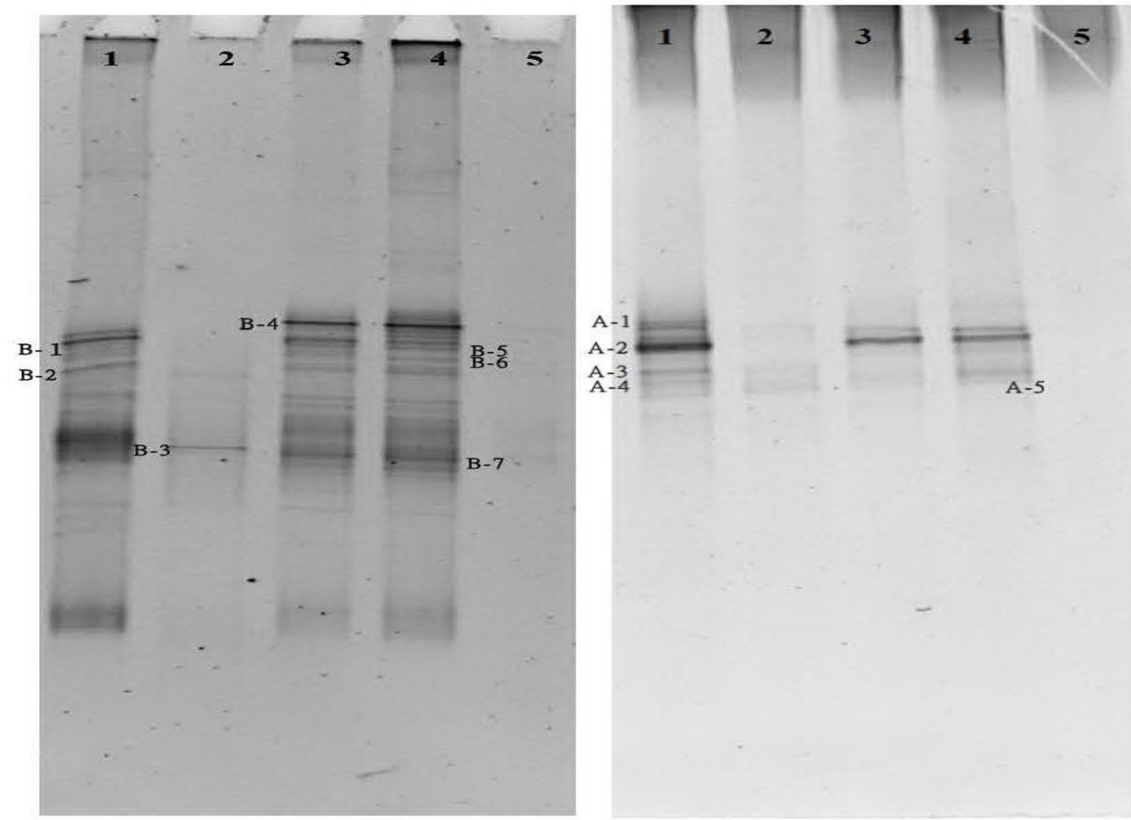


Figure 5. Alkali and trace metals at various BPA added and control reactors



Note:
1 ：968 Control
2 ：968 Anaerobic sludge seeding
3 ：968 BPA10
4 ：968 BPA 100
5 ：968 BPA 500
B-1: *Sporobacterium olearium*
B-2: *Leuconostoc mesenteroides*
B-3: *Clostridiales* sp.
B-4: *Sporobacterium olearium*
B-5: *Clostridium* sp.
B-6: *Klebsiella pneumoniae*
B-7: *Spirochaetaceae* sp.

Note:
1 ：A571 Control
2 ：A571 Anaerobic sludge seeding
3 ：A571 BPA10
4 ：A571 BPA100
5 ：A571 BPA500
A-1: Not specified
A-2: *Methanosarcina* sp.
A-3: Not specified
A-4: *Methanosarcina barkeri*
A-5: Uncultured *Methanomicrobiales archaeon*

Figure 6. DGGE band and corresponding microbial community

PCR and DGGE analyses also showed that *Methanosarcina* sp., *Methanosarcina barkeri* and *Methanomicrobiales archaeon* methane producing bacteria could adapt exposed BPA soluble levels of ~10 - ~30 mg L^{-1} and enhance the biogas production of MSW at BPA 10 and 100 mg L^{-1} daily fed bioreactors between 126 and 305 days' operation.

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