original article

Inhibition and anaerobic biodegradation of benz[*a*] anthracene-7,12- dione via the specific methanogenic activity test

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ABSTRACT

Aims: The purpose of this study was to determine the inhibition and anaerobic biodegradation of benz[*a*]anthracene-7, 12-dione (BaAQ) via the specific methanogenic activity (SMA) test.

Materials and Methods: In this study, 120 mL vials were filled with given concentration of BaAQ, anaerobic biomass and substrate. Each batch experiment was lasted 13 to 26 days. The inhibition effects of BaAQ with concentrations of 0.5, 5, 25, 50, 100, 250 and 500 mg/L on the methanogenic process was investigated in the presence of volatile fatty acids including acetic, butyric and propionic acids.

Results: In some eatch test, the gas production was stopped after 13 days (312 h). Therefore, 312 h was considered as compared base of gas production. After this time, methane production cumulative rate was calculated for the each SMA. After 13 days (312 h) the lowest and the highest cumulative methane has been produced (per mL) at the presence of concentrations of 250 and 0.5 mg/L of BaAQ, respectively.

Conclusion: The results of this study showed that the BaAQ with concentration of 250 mg/L has more inhibition effect on methane production rather than other concentrations (even of 500 mg/L). Therefore, we should not always expect that higher concentrations of toxic compounds had more inhibitory effects than low concentrations of them.

Key words: Benz[*a*]anthracene-7,12-dione (BaAQ), anaerobic biomass, specific methanogenic activity

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INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are "ubiquitous" environmental pollutants.[1-4] These chemical compounds include a group of more than 100 different chemical compounds.[5] These compounds originate from natural and anthropogenic sources[2,6,7] and are formed due to incomplete combustion of organic materials (as natural

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and anthropogenic activities) and are abundant in the environment.[2,8-10] Considering their negative resonance energy and high-melting and boiling point, low solubility in water, and low vaporization pressure,[11] they are thermodynamically stable.[5] These compounds have two or more benzene rings[1,2,12-15] that at least two rings are fused with two adjacent carbons in linear, angular, and cluster arrangements.[13,15] Based on United States Environmental Protection Agency (USEPA), 16 compounds that are shown in Figure 1 are selected as priority pollutants due to their high carcinogenicity and toxicity.[3,16-18] These compounds display toxicity, mutagenicity, and carcinogenicity properties.[2,12,19,20] Based on the reports of National Cancer Institute, benzo[*a*] pyrene (pentacyclic) is a carcinogenetic compound for humans and is in Group 1 of carcinogeneic compounds and benz[*a*]anthracene (4-ring) is in Group 2B and is classified as a possible human carcinogen.[21] Exposure to these compounds may result into various cancers such as kidney, bowel, pancreas, and skin.[22,23] The number of rings and chemical properties of these compounds have important effect on their environmental fate. The persistence of these compounds in the environment is higher with the increasing their molecular weight.[15,24,25] For example, half-life of tricyclic phenanthrene in soil ranges from 16–126 days and half-life of a pentacyclic compound like benzo[*a*]pyrene is over 4 years.[24,25] Although polycyclic aromatic hydrocarbons (PAHs) may undergo adsorption, volatilization, photolysis, and chemical degradation,[2,20] microbial degradation is the major degradation process.[2,19,20,26,27] The rate of biodegradation depends on pH, temperature, oxygen, microbial population, degree of acclimation, availability of nutrients, chemical structure of the compound, cellular transport properties, and chemical partitioning in growth medium.[2] A number of bacterial species known to degrade PAHs are isolated from contaminated soil or sediments. Pseudomonas aeruginosa, Pseudomons fluoresence, Mycobacterium spp., Rhodococcus spp., Paenibacillus spp., are some of the commonly studied PAH-degrading bacteria.[2] Also, it was proved that under anaerobic digestion, the highest removal was for tricyclic aromatic PAHs.[26, 27] In the anaerobic systems, adequately maintenance of the methanogenic bacteria population for a fixed performance is difficult. Various methanogenic bacteria species and their relative population in the reactor biomass, moreover the environmental conditions and operations, are dependent on the characterization of sewage. Therefore, any toxic shock leads in the change of different bacterial species and their relative population that as a result of it, the efficiency of the reactor is affected. The performance of the reactor is determined based on the organic matter removal efficiency, the amount of volatile fatty acids, and the amount of produced biogas. Here the specific methanogenic activity (SMA) test is of great importance. At first, these tests were used mostly for inoculated selected sludge but now these tests are used for many other purposes including the assessment of sludge behavior under the influence of compounds with inhibition potential, gaining degradability rate of various compounds, estimation of the maximum applicability of loading for a definite sludge and assessment of kinetics parameters of the Batch system.[28] It is proved that most of the polycyclic aromatic compounds display average and strong carcinogen metabolites but as most of these metabolites have less carcinogenicity compared to their parent compound; they are less taken into attention.[29] In the first stage of microbial metabolism of benz[a]anthracene, this compound is changed into benz[*a*]anthracene-7,12- dione (BaAQ).[30]

This study aims to determine the inhibition of various concentrations of benz[*a*]anthracene-7,12- dione (BaAQ) on anaerobic biomass via the specific methanogenic activity test (SMA).

MATERIALS AND METHODS

This study was designed as an experimental–interventional test. This study was done by a magnetic stirrer, glass vials, and other experiment equipments [Figure 2]. Vials were 120 mL, of which 90 mL was dedicated to liquid section and 30 mL of the head space was considered for accumulation of biogas. Temperature adjustment was done in a heating device

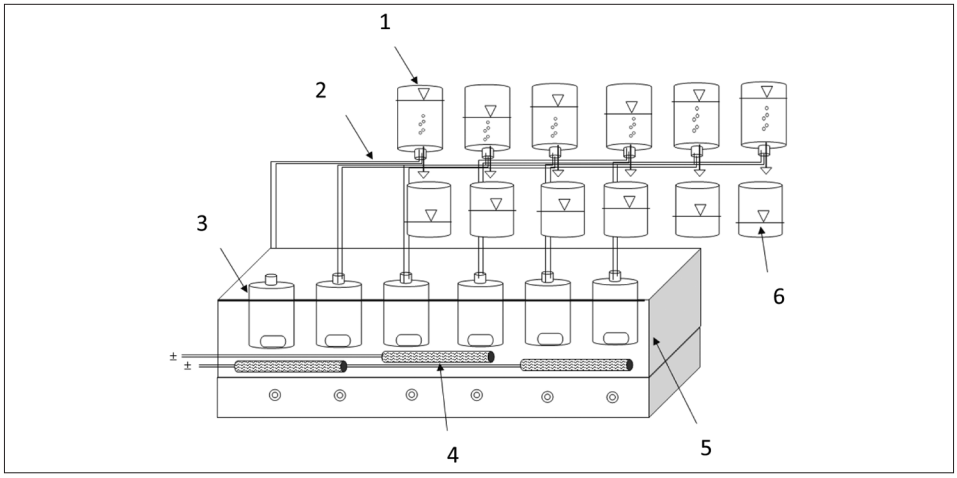

Figure 1: 16 types of PAHs compounds recommended by USEPA as priority pollutants[16]

in which vials were inside it. The materials in the vials were mixed by magnetic stirrer that was under heating enclosure. To pH to pH adjustment and neutralization, specific concentration of the required compound was injected. A magnet starrier was used to complete mixing of vials content. About 45 mL of the washed sludge were poured into each vial and the remain volume of vials was filled with inheritor, substrate and nutrient matter to reach 90 mL. Then, the vials were sealed and placed into hot bath.

In this study, BaAQ concentrations of 0.5, 5, 25, 50, 100, 250 and 500 mg/L were investigated in the presence of volatile fatty acids including acetic, butyric and propionic acids. The produced methane gas data were registered in each day. Performance period of each SMA test took 13 to 26 days. As the gas production of some concentrations was stopped after 13 days (312 h), 13 days was considered as comparison basis of gas production.

The SMA test was investigated to determine the inhibition amount of the mentioned compound. In this test, the ability of anaerobic bacteria was used in production of biogas to determine the inhibition amount of the above compound. To measure the produced biogas in vials, gas replacement with liquid method was used. To do this, biogas outlet pipe that was placed above each of the vials was attached to KOH dish, with a biogas inlet and outlet of KOH. For each produced biogas volume, the same volume of KOH exited and accumulated in a measurement cylinder and was registered as the volume of produced biogas. Figure 1 displays the schematic view of biogas collection system.

RESULTS

As the aim of this study is "determining inhibition and anaerobic biodegradation of benz[*a*] anthracene-7, 12- dione (BaAQ) via the specific methanogenic activity test (SMA)" determining the inhibition amount of these compounds was achieved considering the gas produced of the studied concentrations. It can be said that in some concentrations, gas production was stopped after 13 days (312 h). 13 days was considered as comparison basis of gas production. After the elapsed time for each SMA, methane production cumulative rate was calculated for each SMA and Figures 3–5 were achieved. Methane production cumulative rate (per mL), in the presence of seven various concentrations of BaAQ with the acetic acid as auxiliary substrate, is shown in Figure 3. The lowest and highest amount of the gas after 13 days (312 h) was produced in the presence of concentrations of 250 and 5 mg/L of BaAQ, respectively. Methane production cumulative rate (per mL) in the presence of seven various concentrations of this compound and butyric acid as the auxiliary substrate are shown in Figure 4. The lowest and highest amounts of gas after 13 days (312 h) were produced in the presence of concentrations of 250 and 0.5 mg/L. Methane production cumulative rate, in the presence of seven various concentrations of BaAQ and propionic acid as the auxiliary substrate, is shown in Figure 5. The lowest and highest amount of gas after 13 days (312 h) was produced in the presence of concentrations of 250 and 0.5 mg/L. Methane production cumulative rate (per mL) in the presence of acetic, butyric and propionic acids as the sole carbon source are shown in Figures 3–5, respectively.

DISCUSSION

According to Figures 3–5, the amount of produced methane in all the studied concentrations of BaAQ is more than the concentration of 250 mg/L. Based on these figures, even concentration of 500 mg/L produced more gases. Thus, 250 mg/L concentration of BaAQ with the lowest amount of produced methane was more inhibitor than other concentrations of this compound. It can be said that the possible inhibitors range and their various effects on

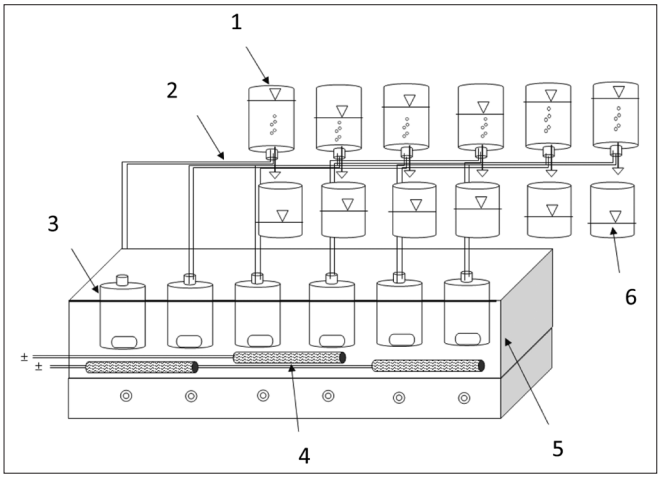

Figure 2: Schematic view of the pilot for the specific methanogenic activity (SMA): 1- CO_2 scrubber (KOH solution), 2- Rubber tubing, 3- Serum bottle, 4- Heater, 5- Hot bath, 6- Displaced liquid

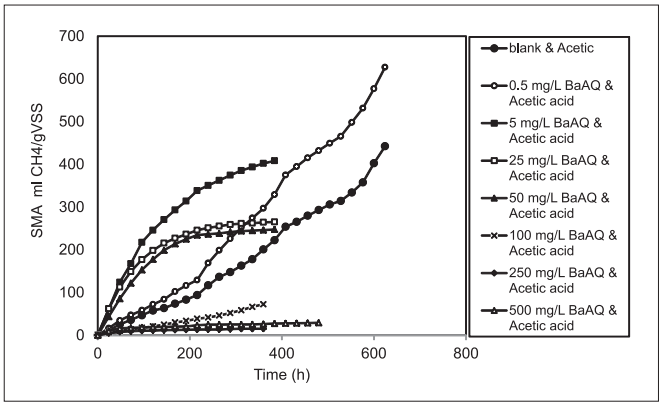

Figure 3: The effect of different concentrations of BaAQ as the main substrate on the specific methanogenic activity of anaerobic biomass in the presence of acetic acid as aid substrate

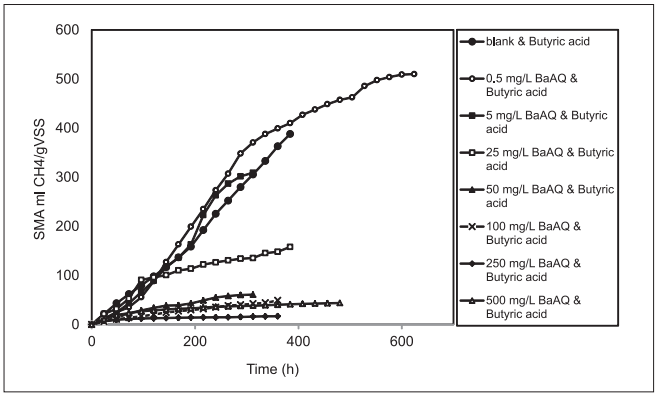

Figure 4: The effect of different concentrations of BaAQ as the main substrate on the specific methanogenic activity of anaerobic biomass in the presence of Butyric acid as aid substrate

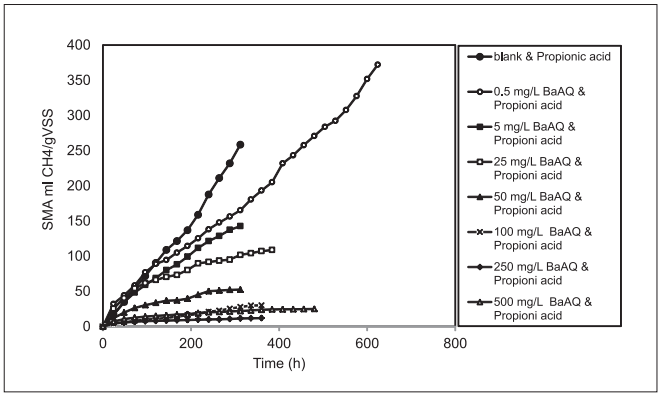

Figure 5: The effect of different concentrations of BaAQ as the main substrate on the specific methanogenic activity of anaerobic biomass in the presence of propionic acid as aid substrate

microorganisms can turn inhibition to a confusing issue. In some cases, inhibitor of an active enzyme affects substrate consumption. In these cases, substrate consumption gets slow. In other cases, the inhibitor affects on a general action of cell-like respiration and indirect effects such as the reduction of biomass concentration can make the consumption of a specific substrate slow. Finally, some of the reactions are promoted by inhibition, because the cell tries to compensate the negative inhibition effect. A common type of inhibition for aromatic hydrocarbons and chlorinated solutes is self-inhibition that is called Haldan or Andros's kinetics. In this case, substrate enzyme degradation is slowly decreased due to the high concentration of the substrate. It is not clear that this self-inhibition is directly conducted because of activities on degradation enzyme or indirectly by affecting electron flux or energy after the reaction of initial donators.[8] Amin *et al*. investigated the effect of erythromycin on the anaerobic sequencing batch reactor (ASBR). After stability of reactor operation conditions, they added the low concentration (1 mg/L) and high concentration (200 mg/L) of erythromycin to the reactor. The results showed that the addition of low amount of erythromycin lead in 5% reduction of biogas production, but high concentration of erythromycin did not have more reduction effect on the biogas production. Thus, it seems that the most of microbial populations in ASBR are resistant against the antibiotic.[31] Recent study (2008) on the biodegradability of oils containing Polychlorinated Biphenyls (PCBs) in the transformers of electricity by anaerobic method in sequencing batch vials(SMA test) showed that PCBs oil in low to average values (0.02 to 0.05 mL) was degraded by microorganisms and by increasing the PCBs oil, the activity of microorganisms is decreased.[32] The study conducted by Camprubi *et al*., showed that some of antibiotics such as chloramphenicol, chlortetracycline, tylosin, and erythromycin have an inhibition effect in the semi-sequencing Batch tests. Among all mentioned antibiotics, chlortetracycline, tylosin, and erythromycin did not have any inhibitory effect on the methanogenic activity but chloramphenicol had inhibitory effects.[33] Generally, it can be concluded that we should not always expect that higher concentrations of toxic compounds had more inhibitory effects than low concentrations of them. Further studies are required to evaluate the remediation of toxic compounds and determination of their inhibitory via the specific methanogenic activity (SMA) test.

CONCLUSIONS

In this study, the inhibition of various concentrations of benz[*a*]anthracene-7,12- dione (BaAQ) on anaerobic biomass via the specific methanogenic activity test (SMA) was investigated. The results showed that the effects of BaAQ with concentration of 250 mg/L in the presence of three volatile fatty acids are more inhibitor than other its concentrations (even of 500 mg/L) for methane production. Therefore, it should not be expected that the inhibitory effects were increased with the increasing toxic compounds. The results obtained in the present study suggested that SMA test is a feasible method for assessing inhibitory concentrations of toxic substances.

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REFERENCES

1. Hamdi H, Benzarti S, Manusadzianas L, Aoyama I, Jedidi N. Bioaugmentation and biostimulation effects on PAH dissipation and soil ecotoxicity under controlled conditions. Soil Biol Biochem 2007;39:1926-35.
2. Haritash A, Kaushik C. Biodegradation aspects of polycyclic aromatic hydrocarbons (PAHs): A review. J Hazar mater 2009;169:1-15.
3. Kumar S, Upadhayay SK, Kumari B, Tiwari S, Singh S, Singh P. *In vitro*

degradation of fluoranthene by bacteria isolated from petroleum sludge. Bioresour Technol 2011;102:3709-15.
4. Orecchio S, Ciotti VP, Culotta L. Polycyclic aromatic hydrocarbons (PAHs) in coffee brew samples: Analytical method by GC-MS, profile, levels and sources. Food Chem Toxicol 2009;47:819-26.
5. Ipcs O. Environmental Health Criteria 202-Selected non-heterocyclic Polycyclic Aromatic Hydrocarbons. World Health Organisation, International Programme on chemical Safety. Available from: http:// www inchem org. [Last accessed on 1998].
6. Mueller JG, Cerniglia C, Pritchard PH. Bioremediation of environments contaminated by polycyclic aromatic hydrocarbons. Biotechnol Res 1996;6:125-94.
7. Wisniowska E, Janosz-Rajczyk M. Selected PAHs concentration changes under nitrate and sulphate reducing conditions. Desalination 2007;211:232-7.
8. Bruce ER, Perry LMC. Environmental biotechnology: Principles and applications. New York: McGraw-Hill; 2001.
9. Khadhar S, Higashi T, Hamdi H, Matsuyama S, Charef A. Distribution of 16 EPA-priority polycyclic aromatic hydrocarbons (PAHs) in sludges collected from nine Tunisian wastewater treatment plants. J Hazard Mater 2010;183:98-102.
10. Meckenstock RU, Safinowski M, Griebler C. Anaerobic degradation of polycyclic aromatic hydrocarbons. FEMS Microbiol Ecol 2004;49:27-36.
11. Nam K, Kukor JJ. Combined ozonation and biodegradation for remediationof mixtures of polycyclic aromatic hydrocarbons in soil. Biodegradation 2000;11:1-9.
12. Dou J, Liu X, Ding A. Anaerobic degradation of naphthalene by the mixed bacteria under nitrate reducing conditions. J Hazard Mater 2009;165:325-31.
13. Doyle E, Muckian L, Hickey AM, Clipson N. Microbial PAH degradation. Adv Appl Microbiol 2008;65:27-66.
14. Fuchedzhieva N, Karakashev D, Angelidaki I. Anaerobic biodegradation of fluoranthene under methanogenic conditions in presence of surface-active compounds. J Hazard Mater 2008;153:123-7.
15. Juhasz AL, Naidu R. Bioremediation of high molecular weight polycyclic aromatic hydrocarbons: A review of the microbial degradation of benzo (a) pyrene. Int Biodeterior Biodegradation 2000;45:57-88.
16. Van Stijn F, Kerkhoff M, Vandeginste B. Determination of polycyclic aromatic hydrocarbons in edible oils and fats by on-line donor-acceptor complex chromatography and high-performance liquid chromatography with fluorescence detection. J Chromatogr A 1996;750:263-73.
17. Bernal-Martinez A, Patureau D, Delgenès JP, Carrere H. Removal of polycyclic aromatic hydrocarbons (PAH) during anaerobic digestion with recirculation of ozonated digested sludge. J Hazard Mater 2009;162:1145-50.
18. Hossain MA, Hoque MZ. Polycyclic aromatic hydrocarbons in Bangladeshi vegetables and fruits. Food Chem Toxicol 2011;49:244-7.
19. Sayara T, Pognani M, Sarrà M, Sánchez A. Anaerobic degradation of PAHs in soil: Impacts of concentration and amendment stability on the PAHs degradation and biogas production. Int Biodeterior Biodegradation 2010;64:286-92.
20. Ambrosoli R, Petruzzelli L, Luis Minati J, Ajmone Marsan F. Anaerobic PAH degradation in soil by a mixed bacterial consortium under denitrifying conditions. Chemosphere 2005;60:1231-6.
21. IARC, Overall Evaluations of Carcinogenicity to Humans, Group 1: Carcinogenic to humans, 2009. Available from: http://www.monographs. IARC. Fr/ENG/Classification/ crthgr01list. Php. [Last accessed on 2008].
22. Samanta SK, Singh OV, Jain RK. Polycyclic aromatic hydrocarbons: Environmental pollution and bioremediation. Trends Biotechnol 2002;20:243-8.
23. Mastrangelo G, Fadda E, Marzia V. Polycyclic aromatic hydrocarbons and cancer in man. Environ Health Perspect 1996;104:1166-70.
24. Dabestani R, Ivanov IN. A compilation of physical, spectroscopic and photophysical properties of polycyclic aromatic hydrocarbons. Photochem Photobiol 1999;70:10-34.
25. Wilcke W. Synopsis Polycyclic Aromatic Hydrocarbons (PAHs) in Soil—a Review. J Plant Nutr Soil Sci 2000;163:229-48.
26. Bernal-Martinez A, Carrere H, Patureau D, Delgenès JP. Ozone pre-treatment as improver of PAH removal during anaerobic digestion of urban sludge. Chemosphere 2007;68:1013-9.
27. Chang B, Chang S, Yuan S. Anaerobic degradation of polycyclic aromatic hydrocarbons in sludge. Adv Environ Res 2003;7:623-8.
28. Amin MM. Performance evaluation of three anaerobic bioreactor: ASBR, HAIS, and UASB, PhD Thesis, School of Health Isfahan University of Medical Sciences Isfahan, Iran. 2004.
29. Horn J, Flesher JW, Lehner AF. The metabolism of formyl-substituted benzanthracenes to hydroxymethyl metabolites in rat liver *in vitro* and *in vivo*. Chem Biol Interact 2003;145:17-32.
30. Cajthaml T, Erbanová P, Sasek V, Moeder M. Breakdown products on metabolic pathway of degradation of benz (a) anthracene by a ligninolytic fungus. Chemosphere 2006;64:560-4.
31. Amin MM, Zilles JL, Greiner J, Charbonneau S, Raskin L, Morgenroth E. Influence of the antibiotic erythromycin on anaerobic treatment of a pharmaceutical wastewater. Environ Sci Technol 2006;40:3971-7.
32. Moradpour H. The study of degradability of PCB oil in transformers of electricity department of Isfahan Zoube Ahan by anaerobic method in sequencing batch vials. MS thesis in environmental health engineering. Iran: Department of Health, Isfahan University of Medical Sciences; 2009.
33. Camprubi M, Paris JM, Casas C. Effects of antimicrobialagents and feed additives on the performance of piggery waste anaerobic treatment. In: Hall ER, Hobson PH, editors. Advances in Water Pollution Control. Anaerobic Digestion. Oxford: Pergamon Press; 1988. p. 239–48.

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