original article

Effects of oxytetracycline, tylosin, and amoxicillin antibiotics on specific methanogenic activity of anaerobic biomass

Mohammad Mehdi Amin, Hassan Hashemi, Afshin Ebrahimi, Asghar Ebrahimi

Environment Research Center and Department of Environmental Health Engineering, Isfahan University of Medical Sciences, Isfahan, Iran

ABSTRACT

Aims: The purpose of this study was to survey the antibiotics effects of oxytetracycline, tylosin, and amoxicillin on anerobic wastewater treatment process.

Materials and Methods: To evaluate the inhibitory antibiotics amoxicillin, tetracycline, and tylosin on biomass activity, specific methanogenic activity (SMA) using anerobic biomass batch; into 120 ml vials: 30 ml biomass and 70 ml substrate including volatile fatty acids, mainly acetic acid and various concentrations of antibiotics were added. Methane gas production replacement through solution of KOH (2 N) as an absorber of CO_2 and bromine thymol blue as indicator was measured. Each batch was tested for 10 days.

Results: Based on the findings, inhibitory concentration of oxytetracycline, amoxicillin, and tylosin were 8000, 9000, and 9000 mg/L, respectively.

Conclusions: This study showed that with increasing concentrations of antibiotics, the produced biogas volume from biomass per unit weight is decreased. COD removal was 42-82 % due to long retention time and adsorption to flocks.

Key words: Antibiotic, oxytetracycline, specific methanogenic activity, tylosin, and amoxicillin

Address for correspondence:
Eng. Hassan Hashemi,
Environment Research Center,
Isfahan University of Medical Sciences, Hezar Jerib Avenue, Isfahan, Iran.
E-mail: hashemi@hlth.mui.ac.ir

INTRODUCTION

There are various antibiotics and drugs in pharmaceutical industry's wastewater depending on the type of produced drugs. Extensive use of these drugs may cause many biological hazards. Since, in addition to their direct presence in the environment, they prevent the effective treatment of wastewater.[1]

Access this article online	
Quick Response Code:	Website: www.ijehe.org
	DOI: 10.4103/2277-9183.102356

The presence of antibiotics and synthetic antimicrobial drugs in pharmaceutical industry's wastewater may affect the biological treatment of such wastewater and also the bioreactor microbial community. However, long contact with these antimicrobial drugs leads to the resistance of micro-organisms, which are effective in biodegradation and the efficiency of biological treatment processes improves.[2]

In this study, the focus is on the three types of antibiotics oxytetracycline, amoxicillin, and tylosin, which are used by human and livestock relatively more than other antibiotics. The effects of these antibiotics on the anerobic treatment of wastewater from pharmaceutical industry and on the methanogens activity or biomass in reactors were studied.[3] Specific methanogenic activity (SMA) is a valid examination

This article may be cited as:
Amin MM, Hashemi H, Ebrahimi A, Ebrahimi A. Effects of oxytetracycline, tylosin, and amoxicillin antibiotics on specific methanogenic activity of anaerobic biomass. Int J Env Health Eng 2012;1:37.

for monitoring the changes in the numbers and activity of methanogenic bacteria in biological treatment of pharmaceutical wastewater in bioreactors.[4]

The effects of antibiotics on the bacteria and their numbers can be examined through measuring the amount of biogas (CH_4) produced by methanogens.[5]

The studied antibiotics have an aromatic structure, which inhibits the activity of bacteria by affecting their RNA. Inhibitory mechanism of these antibiotics is that they cause ribosome 235 methylation (adding CH3) in RNA. The sensitivity of gram-positive bacteria to the antibiotics is more than that of gram-negative bacteria.[6] According to recent studies, a variety of antibiotics exist in surface and groundwater in many countries. These antibiotics do not tend to be absorbed by soil and enter the groundwater through layers of earth. Therefore, it is essential to eliminate antibiotics from pharmaceutical, municipal, agricultural, and industrial wastewater. According to reports from the United States Geological Survey (USGS), the presence of antibiotics in surface water shows its contamination by wastewater causing potential effects on surface water. In these studies, antibiotics were measured in surface water in the concentration of 1.7 μg/l.[7]

Antibiotics including antimicrobial drugs are mainly prescribed in medicine to deal with all kinds of infections. When taking either oral or parenteral antibiotics, it affects the body and then excreted. The amount of absorption and excretion of antibiotic depends on the type of used antibiotic.[8] When antibiotics and its metabolites enter the environment and water resources cause toxic effects on organisms. Tetracyclines are broad-spectrum antibiotics inhibiting protein synthesis and are bacteriostatic for much of gram-negative and gram-positive bacteria including anerobic, rickettsia, Chlamydia, and mycoplasma and act against some protozoa such as amoeba.[9] The effectiveness of these drugs differs due to their different absorption, distribution, and excretion properties. Inside cells, tetracycline reversibly binds to the 50S subunit of bacterial ribosome and inhibits amino acetyl-tRNA binding to receptor sites and ribosomal mRNA complex. This binding prevents the addition of amino acid to the growing peptide. Cell resistance to tetracycline shows its resistance to multiple drugs.[10]

The direct effect of high oxytetracycline (OTC) loadings (155.56 and -177.78 g OTC/m3.d) on acidogens and methanogens were evaluated with Haldane inhibition kinetic. A significant decrease of the Haldane inhibition constant was indicative of increases in toxicity at increasing loading rates. Substantial inhibitory effects on the half velocity constant of molasses-COD concentration (Ks; mg/L) and the Haldane inhibition constant (KID; mg/L) values were observed, as was evidenced by a decrease in KID values for OTC higher than 300 mg/L in the Haldane inhibition model.[11]

Widespread use of tetracycline for mild disease causes resistance among other sensitive groups. Tetracycline is used in animal feed to a large extent to increase their growth, which is involved in the increase of resistance to tetracycline intestinal bacteria including coliforms and in plasmids carrying tetracycline resistance gene.[12] The concentration of tetracyclines in urine becomes more than 300 μg/ml, 2 hours after drug absorption and remains constant until 12 hours after absorption. Tylosin belongs to the microlide group.[9] The objective of this study is to examine the effects of oxytetracycline, tylosin, and amoxicillin antibiotics on SMA of anerobic biomass.

MATERIALS AND METHODS

Glass vials of 120 ml were used to test every batch which lasted 10 days. Due to the relatively small size of vials, it was possible to test 6 vials in each batch and to inject a specific concentration of the antibiotics to each vial. In order to mix the contents of reactor, some magnets were put into each vial making the sludge mix with the substrate and the antibiotics completely with their rotation.

A specific volume of the sludge removed from treatment plant was washed to obtain its special granules of which 15 ml was poured into each vial. Then, a certain concentration of antibiotic was added to the vials after adding a specific amount of substrate and nutrients. The temperature was kept at about 32°C.

For the better activity of anerobic biomass and better performance of methanogens, a substrate mixture of macronutrients and micronutrients was added to the reactor. The reactor was fed with synthetic wastewater with chemical oxygen demand (COD) of 1625 mg/l. various compounds of the substrate were shown in Table 1. Furthermore, 36 mg/l of yeast extract and 36 mg/l of peptone were added to the reactor.[13]

Oxytetracycline and tylosin containing 200 mg of the effective antibiotic material and amoxicillin capsule containing 200 mg of amoxicillin in different concentrations were tested. pH of the reactor was adjusted with KOH (1:1 molar ratio). During 10 months of the reactor operation, the methane gas produced

Table 1: The compounds of substrate used in SMA test

Inorganic nutrients	COD, mg/l
Acetic acid	76.45 COD
NH_4Cl	10 mg/g COD
KH_2PO_4	10 mg/g COD
Trace elements	
K_2HPO_4	25.32 mg/g COD
Fe Cl_3	1.021
$Cacl_2$, $2H_2O$	2.06
$MgSO_4$, 7 H_2O	2.14
$MnCl_2$, 2 H_2O	0.34
$CoCl_{2,\ 6}$ H_2O	0.092
$NiSo_4$, 6 H_2O	0.0793
$ZnSo_4$	0.0592
$NaMoO_4$, 2 H_2O	0.0822
$CuCl_2$, 2 H_2O	0.016
HBO_3	0.020

from the anerobic biomass activity was measured daily by replacing the gas with KOH (2 N) solution as an absorber of CO_2 and using bromine thymol blue as an indicator. Considering a 10-day period for each batch testing, the effect of different concentrations of oxytetracycline, amoxicillin, and tylosin on biomass ativity was studied. Pure anerobic biomass activity and its methanogenic capability were studied in separate tests and the volatile suspended solids were determined in the laboratory. The anerobic biomass methanogens activity was examined while no subatrate or antibiotic was added to the reactor.

The schematic of SMA test in bench scale was shown in Figure 1.

RESULTS

In further experiments, some prepared substrate (about 5 ml) and 0.7 g of glucose were added to the biomass. As expected, this time methanogens were enhanced and the methane gas increased compared to the previous experiments without substrate. The pure volatile suspended biomass solids that entered the reactor was measured. Regarding the biomass capability in producing methane gas and also the enhancement of the biomass with certain amount of nutrients, various concentrations of antibiotics were added in further processes. These processes started with adding low concentration and continued with higher concentrations in further tests. The effect of 10,000 mg/l concentration of antibiotic on anerobic biomass activity was quite inhibitory, therefore, the experiments started with low concentrations until it reached the desired extent. The lowest concentration of the added antibiotic was 10 mg/l for the three studied antibiotics. Methanogens were tolerant to low concentrations and continued their activity easily. However, during the first days of the experiment, due to the compatibility of methanogens with the environment, the amount of produced gas was low and scarcely increased until it reached the pick point and then decreased again. A similar study on the effect of erythromycin on anerobic biomass showed that the gas production and COD removal decreased to 5-10% and methanogens activity decreased from 3.64 to 0.64 ml/gVSS.d when erythromycin was added to the biomass. Moreover, in this study, the antibiotics removal was 42-82% due to the long retention time and adsorbing to the flocks. Results on measurement of daily produced methane gas (ml/grVSS per day) showed that all the three antibiotics had rather similar effects on methanogens activity in anerobic biomass. The results by COD test before and after each test showed that COD decreased to 10-15% when the antibiotics were injected to the reactor. The process of biogas production while adding various antibiotics was shown in Figures 2-4.

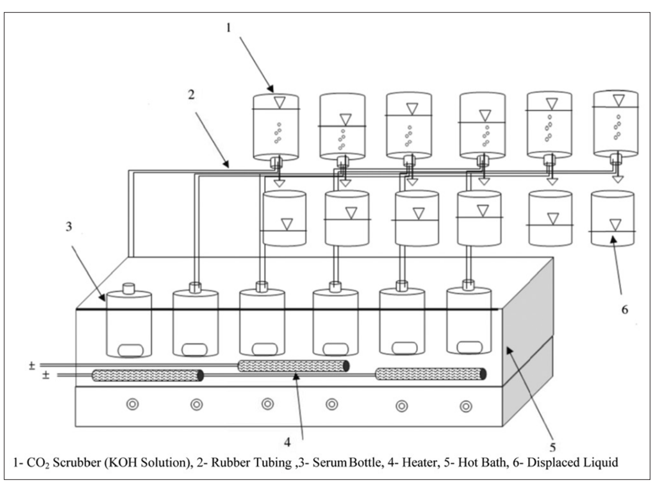

Figure 1: Schematic of SMA test in bench scale;1- CO_2 Scrubber (KOH Solution), 2- Rubber Tubing ,3- Serum Bottle, 4- Heater, 5- Hot Bath, 6- Displaced Liquid

DISCUSSION

Antibiotics disturb the gas production during anerobic wastewater treatment processes. Antibiotics with low concentrations act as bacteriostatics stopping methanogens activity and make the produced methane gas have a downward trend. Antibiotics with high concentrations act as bacteriocides stopping the methanogenic bacteria activity completely and methane gas production reaches zero. In alkaline pH regions, the antimicrobial effect of antibiotic increases due to the increase in non-ionizing part of drug, therefore, biogas production decreases in alkaline pH region. Antibiotics disturb and inhibit the performance of bacteria degrading volatile fatty acids.

Oxytetracycline is a very strong inhibitor in anerobic digestion, stopping the methanogenic bacteria activity completely.

Oxytetracycline inhibits the methanogenic bacteria activity by affecting cell wall, while amoxicillin and tylosin affect the ribosomal 50S subunit. Therefore, lower levels of

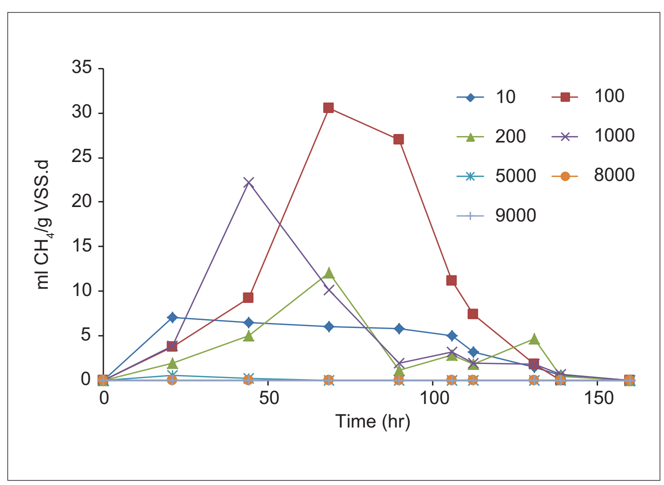

Figure 2: Specific methanogenic activity while adding oxytetracycline

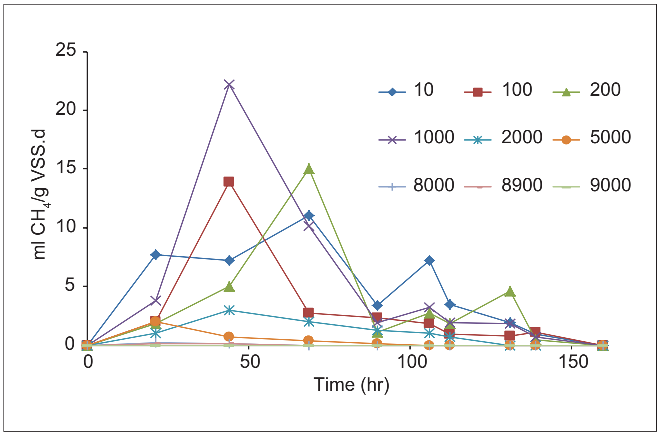

Figure 3: Inhibition effects of amoxicillin on methane production

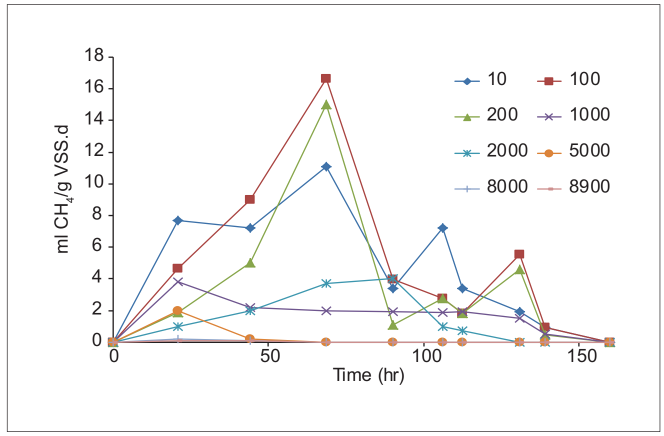

Figure 4: Influence of tylosin on SMA

oxytetracycline are needed to stop the methanogenic bacteria activity compared to amoxicillin and tylosin.

Tetracycline is absorbed by clay, soil, and sediments and cannot move easily. Therefore, it makes disturbances in COD removal when absorbed by flocks in the reactor.[9] In this respect, the COD of the reactor's output wastewater contains more oxytetracycline. When the concentration of antibiotic increases, COD removal decreases and so its output amount increases.[13,14] As the absorption of tylosin and amoxicillin by sediments is much less than that of oxytetracycline, COD removal in reactors containing these two antibiotics is more than other antibiotics, therefore, less COD is observed in wastewater reactors.

Cumulative methane production of 100, 500, and 1000 mg/l of Gentamicin using acetic acid as co-substrate were: 141, 204, and 257 mL, respectively. These rates for 200, 500, and 1000 mg/L of Ampicillin were, 66, 101, and 154 mL, respectively.[15] In this study, cumulative methane production (mL) for 10, 100, 200 and 1000 mg/l of oxytetracycline were 35, 91, 30, and 46; for amoxicillin were 43, 25, 33, and 46; and for tylosin were 43, 45, 33, and 16 using acetic acid as co-substrate, respectively.

According to the results obtained from each batch test, inhibitory concentration of oxytetracycline, amoxicillin, and tylosin were 8000, 9000, and 9000 mg/L respectively.

ACKNOWLEDGEMENT

The authors would like acknowledge the vice-chancellery of research and technology, in Isfahan University of Medical Sciences due to granting the Research Project #284030.

REFERENCES

1. Tchobanoglous G, Burton F L, Stensel D H, Wastewater Engineering: Treatment and Reuse, Mc Graw Hill, New York, 2003:505-957.
2. Eckenfelder W W, Industrial water pollution control, Mc Graw Hill, New York, 2000:70-76.
3. Hugo W B, Russell A D. Pharmaceutical Microbiology. John Wiley & Sons. New York, 1998:401-20.
4. Adams C, Wang Y, Loftin K, Meyer M. Removal of antibiotics from surface and distilled water in conventional water treatment processes. J. Environ. Eng. 2002;128(3):253-60.
5. Kim YH, Heinze TM, Kim SJ, Cerniglia CE. Adsorption and clay-catalyzed degradation of erythromycin A on homoionic clays. J Environ Qual. 2004;33(1):257-64.
6. Martindale W. Martindale: The extra pharmacopeia, 28th Ed. Wiley-Liss, Inc., A Wiley, London, 1984:524-527.
7. Tao R, Ying GG, Su HC, Zhou HW, Sidhu JP. Detection of antibiotic resistance and tetracycline resistance genes in Enterobacteriaceae isolated from the Pearl rivers in South China. Environmental Pollution 158,2010: 2101-2109.
8. APHA/AWWA/WEF. Standard methods for the examination of water and wastewater, 20 th ed., Washington, DC. 2005.
9. EPA. Fate, Transport and Transformation Test Guidelines OPPTS 835.3420 Anaerobic Biodegradability of Organic Compounds in Digested Sludge: By Measurement of Gas Production, National Service Center for Environmental Publications (NSCEP) 1998.
10. Jawed M, Tare V. Microbial composition assessment of anaerobic biomass through methanogenic activity tests, Water SA (1999); 25(3): 345-350.
11. Sponza DT, Ã‡elebi H. Removal of oxytetracycline (OTC) in a synthetic pharmaceutical wastewater by a sequential anaerobic multichamber bed reactor (AMCBR)/completely stirred tank reactor (CSTR) system: Biodegradation and inhibition kinetics. Bioresour Tech. 2012;104:100-10.
12. Dong Jun-ling, Zhao Ying (Lianyungang Environmental Monitoring Central,Lianyungang,Jiangsu 222001,China);A case Study on Pharmaceutical Wastewater Treatment; J Pollution Control Technology.2008:4.
13. 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.
14. Upadhyay R, Mishra N. Antimicrobial Activity of Flower Extracts of Coli Forms Sphaeranthus Indicus on. J Exp Biol Sci 2011;2:513-16.
15. Heidari M, Nabavi BF, Khuzani SF, Amin MM. Inhibition effects of ampicillin and gentamicin by SMA test. Int J Env Health Eng 2012; 1(3):27.

Source of Support: Isfahan University of Medical Sciences, **Conflict of Interest:** None declared.
This article has been previously published in a local Journal in Persian language.