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Inhibition effects of antibiotics ampicillin and gentamycin on the methanogenic activity of anaerobic biomass

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ABSTRACT

Aims: Inhibition behavior of two types of antibiotics including Ampicillin and Gentamicin on specific methanogenic activity of anaerobic biomass has been investigated.

Materials and Methods: A total of 18 Specific Methanogenic Activity (SMA) tests were conducted in 120-ml vials containing 40 v/v% substrate, 37 v/v% biomass and 23 v/v% biogas in batch mode for 20-25 days. Produced methane was measured by gas replacement with 2N KOH solution as CO_2 absorbent. Three volatile fatty acids (VFAs) including acetic acid, propionic acid, and butyric acid were used as co-substrate.

Results: In the tests with 200, 500 and 1000 mg/l of ampicillin at presence of acetic acid, the cumulative SMA were 66, 101, and 154 ml CH_4/g VSS.d, those of with propionic acid were 25, 35, and 46 ml CH_4/g VSS.d, and with butyric acid the values of 198,140, and 245 ml CH_4/g VSS.d were obtained respectively. In the experiments with 100, 500 and 1000 mg/l of gentamicin the cumulative SMA were 141, 204, and 257 ml CH_4/g VSS.d for acetic acid as a substrate, 54, 72 and 71 ml CH_4/g VSS.d for propionic acid, and 139, 74, and 85.5 ml CH_4/g VSS.d for butyric acid, respectively.

Conclusion: At the same concentrations, ampicillin showed more inhibitory effect than gentamicin on anaerobic decomposition of biomass. Within the used VFAs, the inhibitory effects of propionic acid was greater than acetic acid and butyric acid.

Key words: Ampicillin, antibiotic, gentamicin, specific methanogenic activity, volatile fatty acids

INTRODUCTION

Nowadays, apart from pesticides, industrial chemicals, xenobiotic (anthropogenic) organic chemical the-issues of pharmaceutical compounds and their metabolites in the environment has raised increasing concern.[1-3] Pharmaceutical compounds inhibit the wastewater treatment effectiveness, and wide application of these drugs lead to increased environmental risks. Direct presence of these compounds in the wastewater could affect biological treatment processes and the microbial population of bioreactors. Residues of antibiotics and their metabolites in sludge can also negatively influence the treatment systems such as anaerobic digester and nitrification systems.[4]

In anaerobic systems, where there is several ways of biodegradation, short-term inhibition of specific microbial populations cannot result in significant decrease in biogas

Access this article online

Quick Response Code:

Website: www.ijehe.org

DOI: 10.4103/2277-9183.99318

This article may be cited as:
Heidari M, Nabavi BF, Khouzani HS, Amin MM. Inhibition effects of antibiotics ampicillin and gentamycin on the methanogenic activity of anaerobic biomass. Int J Env Health Eng 2012;1:27.

production. However, long term exposure with antimicrobs may cause by- products collection or change in microbial population that can have negative effect on the efficiency of anaerobic treatment.[5]

Camprabi *et al.* (1988) studied the inhibitory effects of some antibiotics such as chloramphenicol, chlorotetracycline, tylosin and erythromycin on the methanogenic activity by batch tests. Chlorotetracycline, tylosin and erythromycin had no have been inhibitory effect, however chloramphenicol has performed deterrent effect.[6]

Sankveist *et al.* (1984) suggested that antibiotics themselves may not prevent the bacterial activity but their metabolites inhibit the bacteria activity in the gastrointestinal tract. Therefore, to evaluate the effect of an antibiotic on anaerobic digestion process, it should be fed to animals rather than directly be added into the mud slurry.[7]

Amin *et al.* (2006) have investigated the effect of erythromycin on the anaerobic sequencing batch reactor (ASBR). They have added low concentration (1 mg/L) and subsequently high concentrations (200 mg/L) of erythromycin into the reactor, after the stabilization of operating conditions. Low concentration of erythromycin led- to 5% decrease in biogas production, but its high concentration did not cause greater reduction of biogas, suggesting that a substantial fraction of the microbial populations in the ASBR showed resistance to the antibiotics. It is because homoacetogenic bacteria which utilized the acetate, are affected by erythromycin.[5]

Poels *et al.* (1984) found that digestion was not disturbed after adding chlorotetracycline, tylosin, erythromycin and chloramphenicol. In contrast, the application of three doses of bacitracin and virginiamycin has caused much decrease in the biogas production. In these experiments, the decrease of gas production was linked to the increase of VFA concentration and soluble COD.[8]

VFAs play an important role in the methane production and their concentrations affect the efficiency of fermentation. However, their effects have not been extensively studied on the yield of methane and growth of methanogenic bacteria. Before being degraded to methane, all VFAs are first degraded to acetic acid and conversion rate of butyric acid to methane is more than propionic acid.[9]

Yeole *et al.* (2009) found that with a propionic acid concentration of 5000 mg/L and pH 7, the methane yield was decreased to 22–38%. And indicated that the inhibitory will increase when pH was decreased.[9]

Yenigun and Demirel (2002) concluded that propionic acid would inhibit the methanogenic bacteria growth when its concentration was more than 951 mg/L. However, adding butyric acid can improve the inhibition to some extent.[9] Yuanyuan *et al.* (2009) found that propionic acid is the main effective substrate for VFAs degradation. They also concluded that increasing of acetic acid and butyric acid concentration accelerate the anaerobic bacteria growth and thus facilitate the conversion of VFAs to acetic acid. In contrast, increasing of propionic acid concentration prevents anaerobic bacteria growth so that VFAs degradation to acetic acid is stopped leading to reduction of methanogenic bacteria activity. They found by statistical analysis that methanogenic bacteria growth rate have a negative correlation with propionic acid concentration ($P < 0.05$) and a positive correlation with butyric acid ($P = 0.01$-0.05) but have no correlation with acetic acid and ethanol concentrations ($P < 0.05$).[9]

In the process of anaerobic digestion, organic loading must be controlled in order to avoid VFAs accumulation and acid fermentation in the system. Other organic acids are degraded more slowly to methane than acetic acid.[10]

Specific methanogenic activity (SMA) test is a safe experiment for monitoring the anaerobic bacteria activity during biological treatment of pharmaceutical effluents in bioreactors. SMA test is used to assess the inhibitory effects of various compounds,[11] but there are little research on its application for the effects of antibiotics and hormones in the pharmaceutical wastewater.

The purpose of this study was to investigate the influence of two antibiotics (ampicillin and gentamicin) in specific methanogenic activity (SMA), which affects the biological treatment efficiency of anaerobic microorganisms in municipal wastewater treatment plants and pharmaceutical industries.

MATERIALS AND METHODS

SMA Test

A total of 18 Specific methanogenic activity (SMA) tests

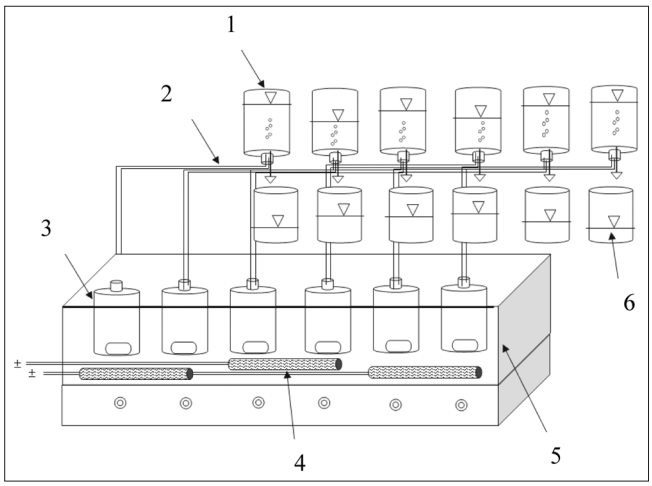

Figure 1: Schematic of methanogenic activity test set-up: 1- CO_2 scrubber (KOH solution); 2- rubber tubing; 3- serum bottle; 4- heater; 5- hot bath, and 6- displaced gas with liquid

were conducted in 120-mLvials in batch mode for 20-25 days. [Figure 1] shows schematic diagram of batch setup used in this study.

In each vial, substrate, biomass and biogas were occupied 48, 45, and 27 ml of volume, respectively. The bottles were capped with butyl rubber stoppers crimped with an aluminum seal. Then the serum bottles were incubated at 35°C in a six places magnetic shaker (Pars Azma Company, Iran) with 20-30 rpm.[11]

Produced methane was measured by gas replacement with 2N KOH solution as a CO_2 absorbent and bromothymol blue as an indicator.[11] The vials were inoculated by anaerobic digester sludge, at rate of 56 and 35 g/l for SS and VSS, respectively.

Substrate

Short chain volatile fatty acids including acetic, butyric and propionic acids were used as substrate for antibiotics [Table 1].

Main substrate is consist of VFAs, antibiotics, nutrients and trace elements and adjusted to neutral pH by addition of NaOH and KOH (1:1 molar ratio). In this study, two types of antibiotics with widely application in medicine were used. Ampicillin (Farabi, Iran) was provided as a powder with purity of more than 97% in 20 g vials and applied at concentrations of 200-1000 mg/L. And gentamicin was applied with a concentration of 100-1000 mg/L.

RESULTS

Table 2 shows different concentrations of used compounds with the maximum cumulative methane production and the concentrations of volatile fatty acid.

The effects of different concentrations of ampicillin and gentamicin antibiotics with using one type of VFA on the cumulative amount of produced methane are shown in [Figures 2-4].

The effects of the same concentration of ampicillin and gentamicin antibiotics against three types of VFA on the cumulative amount of produced methane are shown in [Figures 5-7].

DISCUSSION

Ampicillin with concentrations of 200-1000 mg/L has increased methane production in anaerobic sludge

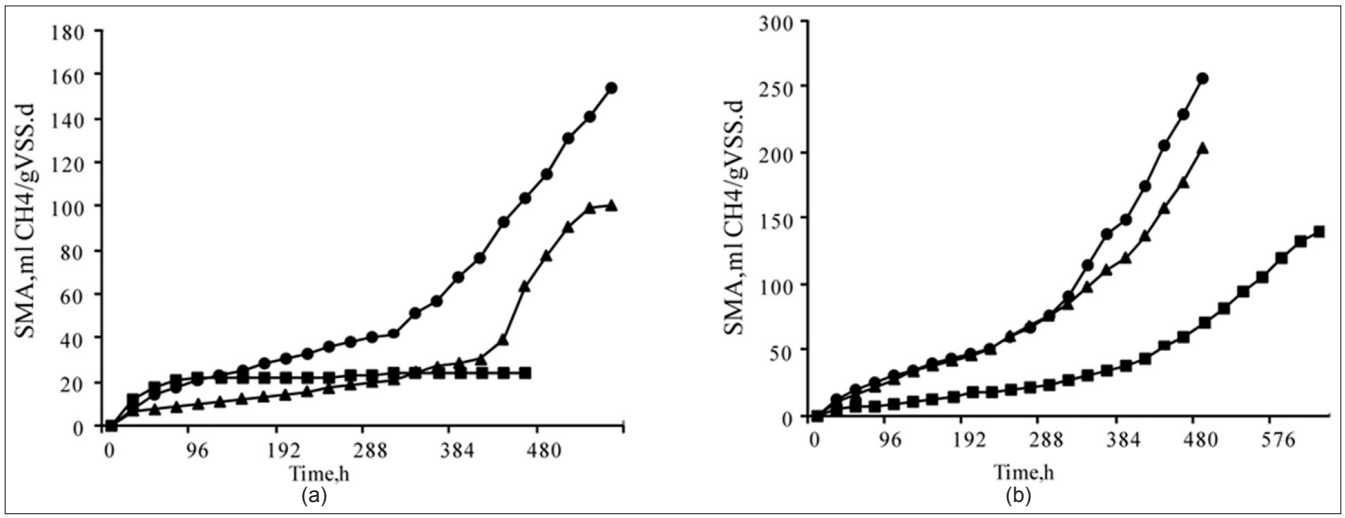

Figure 2: Influence of ampicillin and gentamicin antibiotics on cumulative rate of SMA: (a) different concentrations of ampicillin: 200 (¾), 500 (▲), 1000 (•) mg/L; and (b) gentamicin: 100 (¾), 500 (▲), 1000 (•) mg/L; (Note: acetic acid was used as co-substrate)

Table 1: Specification of co-substrates and used solvent in this study

Antibiotics(mg/L) Ampicillin	COD (mg/L)	COD of VFAs (mg/L)	Volume of VFAs (ml)		
			Propionic Acid	Butyric Acid	Acetic Acid
200	328	46790	3.13	4.18	2.68
500	802	41980	2.81	3.75	2.4
1000	1604	33960	2.27	3.03	1.95
Gentamicin					
100	164	48360	3.23	4.32	2.77
500	822	41780	2.8	3.73	2.4
1000	1644	33560	2.24	2.99	1.92

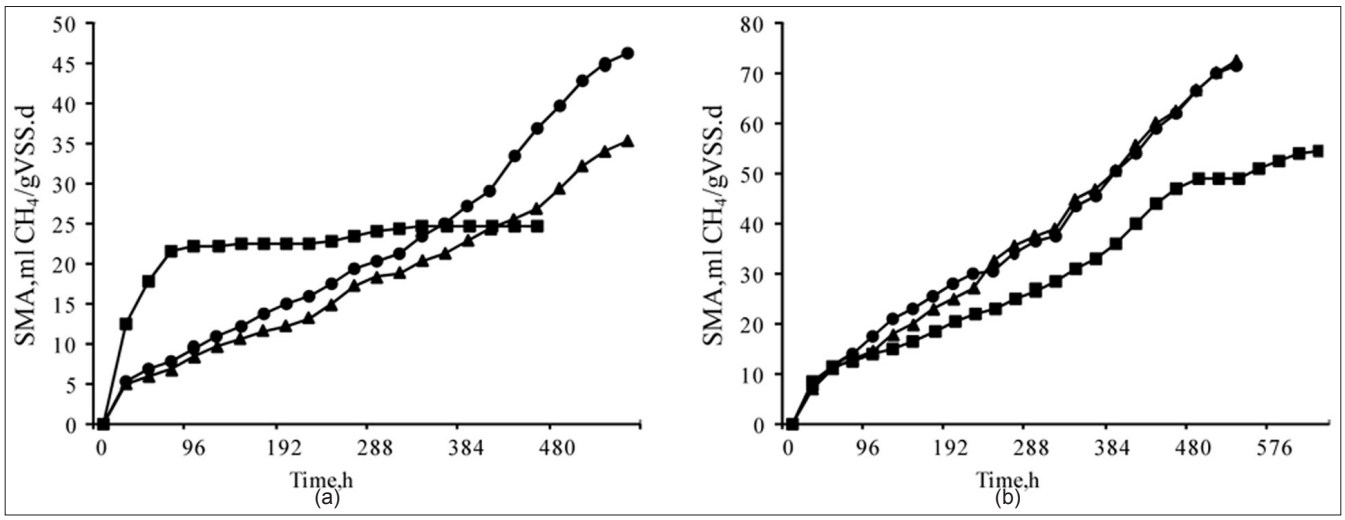

Figure 3: Influence of ampicillin and gentamicin antibiotics on cumulative rate of SMA: (a) different concentrations of ampicillin: 200 (■), 500 (▲), 1000 (•) mg/L; and (b) gentamicin: 100 (¾), 500 (▲), 1000 (•) mg/L; (Note: propionic acid was used as co-substrate)

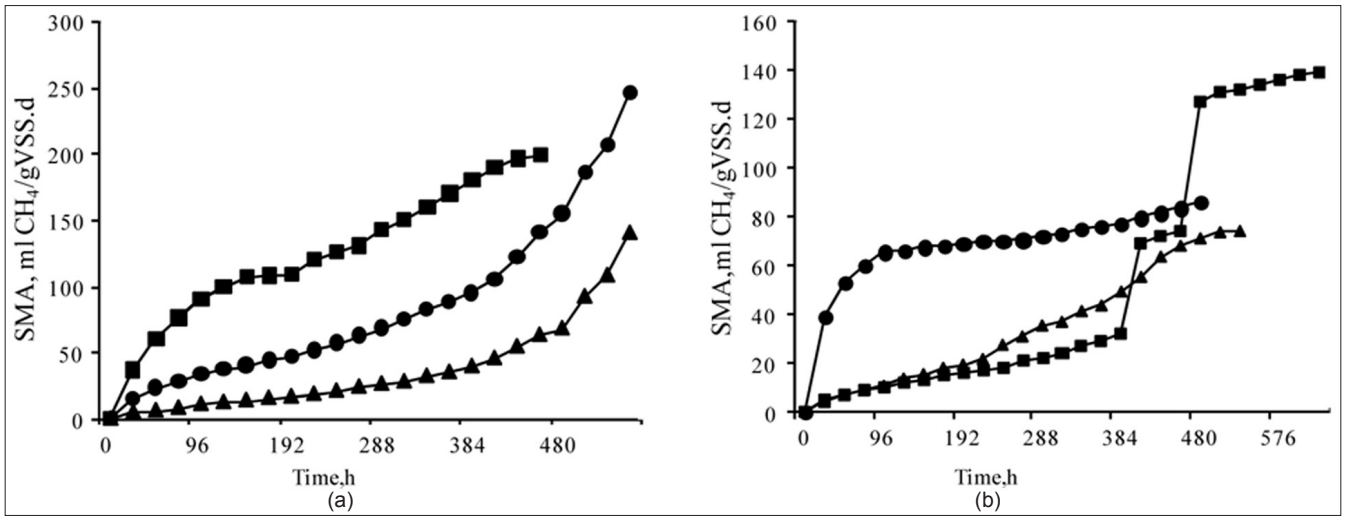

Figure 4: Influence of ampicillin and gentamicin antibiotics on cumulative rate of SMA: (a) different concentrations of ampicillin: 200 (■), 500 (▲), 1000 (•) mg/L; and (b) gentamicin: 100 (■), 500 (▲), 1000 (•) mg/L; (Note: butyric acid was used as co-substrate)

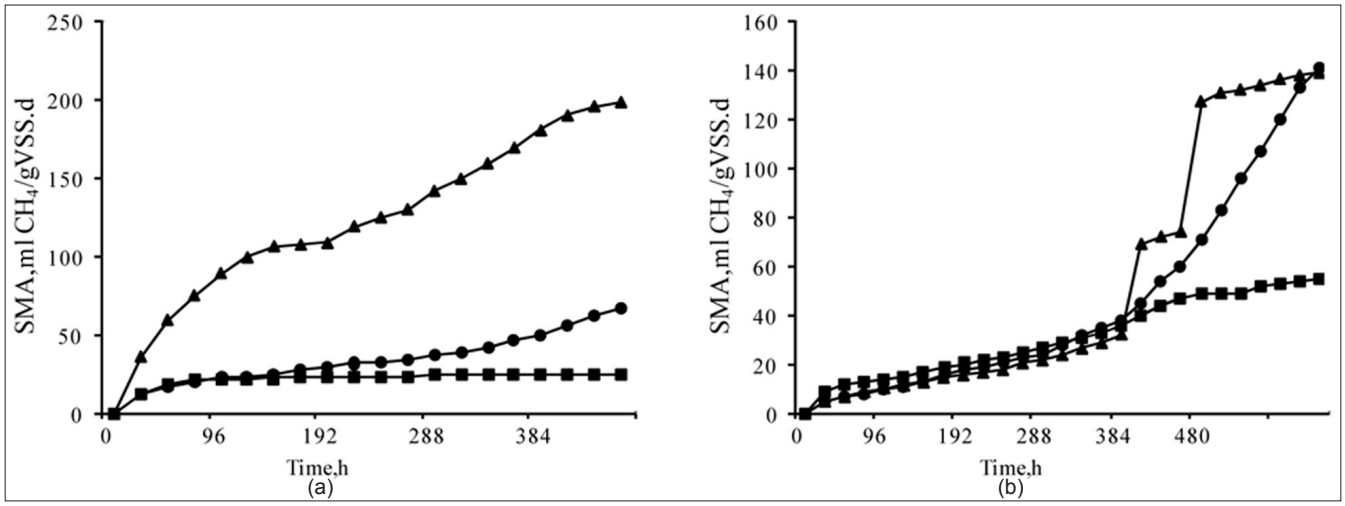

Figure 5: Influence of 200 mg/L ampicillin (a) and 200 mg/L gentamicin (b) on cumulative rate of SMA at presence of three examined volatile fatty acids: acetic acid (•), butyric acid (▲), propionic acid (■)

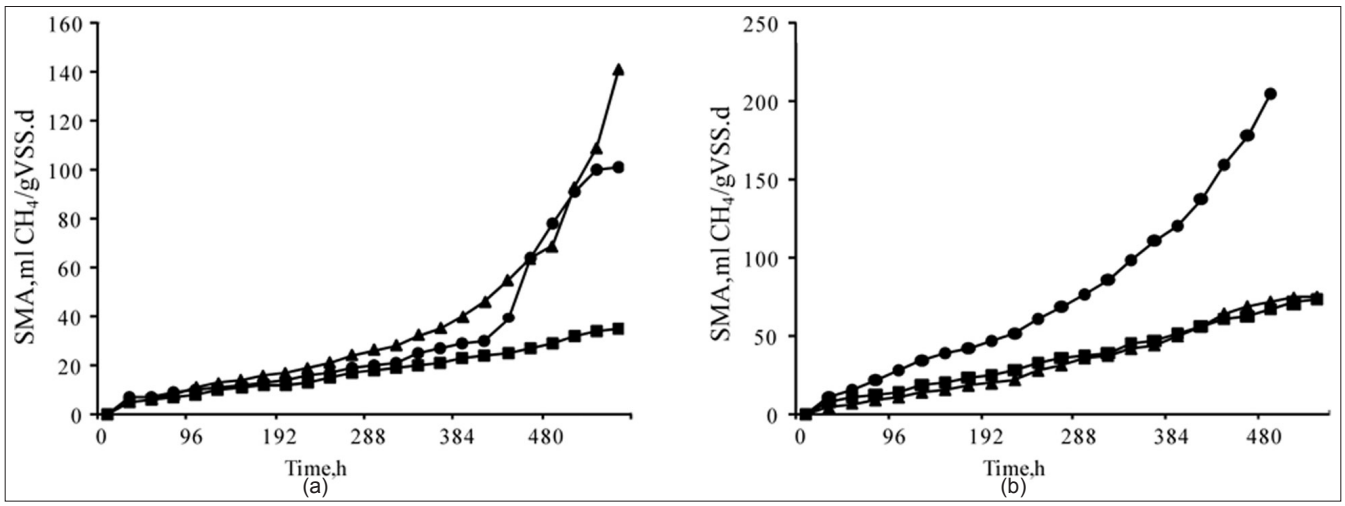

Figure 6: Influence of 500 mg/L ampicillin (a) and 500 mg/L gentamicin (b) on cumulative rate of SMA at presence of three examined volatile fatty acids: acetic acid (•), butyric acid (▲), propionic acid (■)

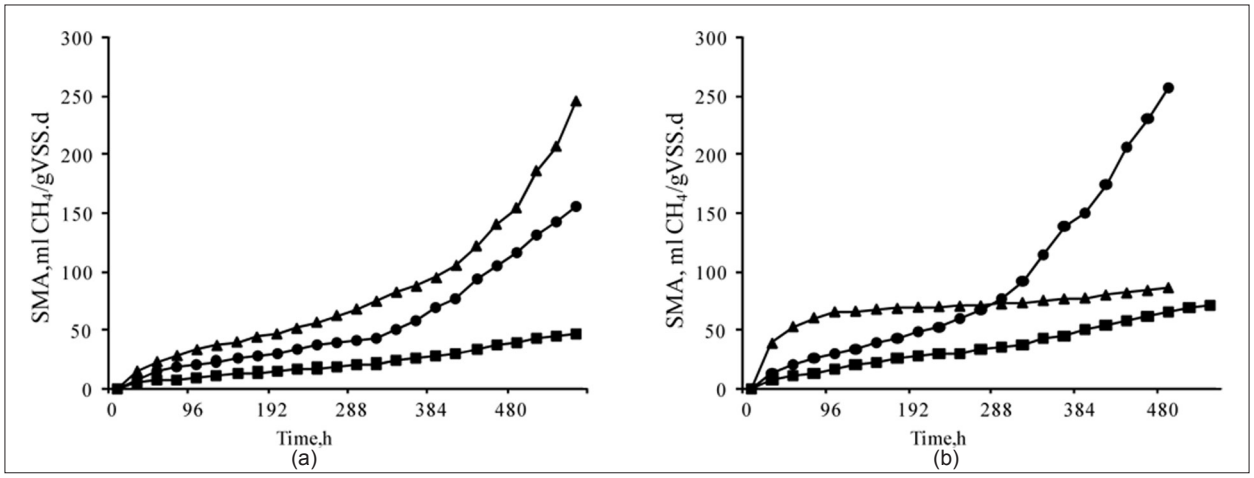

Figure 7: Influence of 1000 mg/L ampicillin (a) and 1000 mg/L gentamicin (b) on cumulative rate of SMA at presence of three examined volatile fatty acids: acetic acid (•), butyric acid (▲), propionic acid (■)

Table 2: Cumulative methane production rate and concentration of volatile fatty acids in the vials containing gentamicin and ampicillin

Antibiotics (mg/L)	Cumulative methane production (ml)			Concentration of VFAs (mg/L)		
	Propionic Acid	Butyric Acid	Acetic Acid	Propionic Acid	Butyric Acid	Acetic Acid
Gentamicin						
100	54	139	141	32042	26592	45360
500	72	74	204	27776	23040	39165
1000	71	85.5	257	22221	18432	31395
Ampicillin						
200	25	198	66	31050	25728	43890
500	35	140	101	27875	23040	39375
1000	46	245	154	22518	18720	31815

[Figures 2a, 3a, and 4a]. This can be demonstrated that, consumption of fatty acid, especially propionic acid, was reduced with increasing concentration of ampicillin and also the inhibitory effect was less.

Methane production increased with increasing concentrations of gentamicin in the range of 100-1000 mg/L and at presence of acetic acid, while, at presence of butyric and propionic acids its production reduced. However, it did not follow a clear trend [Figures 2b, 3b, and 4b].

The rate of methane production in the same concentration of ampicillin and gentamicin on three VFAs are shown in [Figures 5-7]. Propionic acid at presence of the two antibiotics of showed more inhibitory effect on the anaerobic specific methanogenic activity compared to butyric acid and acetic acid.

Also, gradual increase in methane production was observed during 552 hours, which indicates adaptation of bacteria with the anaerobic sludge and their resistance against antibiotics.

The concentration of tested antibiotics versus maximum cumulative methane production and consumed fatty acid are shown in Table 2. The amount of methane production at presence of ampicillin was less than that with gentamicin indicating that ampicillin has more inhibitory effect than gentamicin [Table 2].

In addition, when propionic acid was used, the rate of methane production was decreased compared to the other two fatty acids. If pH be maintained in near-neutral value, volatile acids such as acetic acid or butyric acid have little toxicity on methanogens; however propionic acid is toxic for them.[12] As a result, propionic acid has inhibition effect on specific methanogenic activity.

With increasing concentration of gentamicin from 100 to 1000 mg/L, for acetic acid, the rate of gas production increased, but in the case of butyric and propionic acids regular effect was not observed [Table 2].

The results of this study are comparable with the findings of Yenigun *et al.*, (2002) and Yeol *et al.*, (1996) where in which at pH 7 and propionic acid concentration of 5000 mg/ L. Methane yield decreased to 22 to 38% and they found that at low pH, the inhibition would be increased.[9]

The findings confirm further the results that have been obtained by Demirel and Yenigun (2002). They stated that high concentration of propionic acid e.g., above 951 mg/L would inhibit the methanogenic bacteria growth. Adding butyric acid can also hinder its inhibition to some extent.[9]

CONCLUSION

It is concluded that ampicillin has more inhibitory effect on SMA than gentamicin at similar concentrations. Methane production rate was increased with increasing of ampicillin concentration. In tests with varying concentrations of the two antibiotics propionic acid has more inhibitory effect than butyric acid and acetic acid.

It is suggested that the entrance of the studied antibiotics to full scale treatment systems may caused interference in the methanogenic activity of anaerobic digester of municipal wastewater treatment plants and anaerobic reactors in waste treatment of pharmaceutical industries.

ACKNOWLEDGEMENT

The authors would like acknowledge the vice-chancellery of research and technology, Isfahan University of Medical Sciences due to granting the research project #287301.

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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.