AGRICULTURAL BIOLOGY,
2018, V. 53, № 1, pp. 209-217
(SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA)

ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

UDC 628.355:628.385:573.6.086.835:582.663.2

doi: 10.15389/agrobiology.2018.1.209eng
doi: 10.15389/agrobiology.2018.1.209rus

MATERIALS DERIVED FROM *Amaranthus cruentus* L. USED AS CO-SUBSTRATES CAN INTENSIFY METHANOGENESIS DURING BIOCONVERSION OF ORGANIC WASTE

S.T. MINZANOVA[1], V.F. MIRONOV[1], D.E. BELOSTOTSKII[1], A.Z. MINDUBAEV[1], L.G. MIRONOVA[1], M.S. GINS[2], V.K. GINS[2], P.F. KONONKOV[2], V.A. MILYUKOV[1]

[1]*Arbuzov Institute of Organic and Physical Chemistry, Subdivision of Federal Kazan Scientific Center RAS,* Federal Agency for Scientific Organizations, 8, ul. Arbuzova, Kazan, 420088 Russia, e-mail minzanova@iopc.ru (✉ corresponding author), mironov@iopc.ru, DimBoss@yandex.ru, mindubaev-az@yandex.ru, mironoval1963@gmail.com, miluykov@iopc.ru;
[2]*Federal Research Center for Vegetable Growing,* Federal Agency for Scientific Organizations, 14, ul. Selektsionnaya, pos. VNIISSOK, Odintsovskii Region, Moscow Province, 143080 Russia, e-mail anirr@bk.ru, anirr67@yandex.ru
ORCID:
Minzanova S.T. orcid.org/0000-0001-9678-8821
Mironov V.F. orcid.org/0000-0002-4198-3774
Belostotskii D.E. orcid.org/0000-0002-2824-1223
Mindubaev A.Z. orcid.org/0000-0002-8596-7805
Mironova L.G. orcid.org 0000-0003-1919-571X
Gins M.S. orcid.org/0000-0001-5995-2696
Gins V.K. orcid.org/0000-0002-7053-4345
Kononkov P.F. orcid.org/0000-0001-7101-3528
Milyukov V.A. orcid.org/ 0000-0002-8069-457X

The authors declare no conflict of interests
Acknowledgements:
Supported financially by the RAS Presidium Program № 3 and Russian Foundation for Basic Research (grant № 14-08-31768)
Received January 6, 2017

Abstract

Methane fermentation (biomethanogenesis) performed by a multicomponent microbial consortium under anaerobic conditions results in a mixture of approximately 65 % CH_4, 30 % CO_2, 1 % H_2S and minor amounts of N_2, O_2, H_2 and CO. The peculiarity of biomethanogenesis lies in the ability to convert almost all classes of organic compounds, household, agricultural and some industrial waste into biogas. We were the first to assess the efficiency of the biogas production from organic waste as influenced by various materials derived from amaranth (*Amaranthus cruentus* L.) which were used as co-substrates. Our findings indicate that optimization of the substrate organic matter composition by using dry phytomass of amaranth plants or amaranth pulp which remains after removing all practically valuable substances makes it possible to produce biogas from sewage sludge. This facilitates solving ecological problems of waste disinfection and utilization, and gives us an alternative, cheap and renewable source for fuel. Cultivated *A. cruentus* is a high-yielding protein-rich crop. Its biomass serves as a reproducible raw material. In our previous works, we reported the technology for rutin, vegetable protein and pectin production from *A. cruentus* plants, and suggested a scheme for complex processing which includes extraction of these substances from amaranth dry phytomass in a single technological cycle. The pulp obtained after extraction of all valuable compounds was proposed as a co-substrate for organic waste anaerobic fermentation. We modeled the effect of amaranth-derived substances on biogas production in the laboratory bioreactor using large-tonnage urban sewage sludge as a substrate. It was shown that the doses of the additives affected the process, i.e. the excess of amaranth plant mass (74 % and 87 %) suppressed methanogenesis. The thermophilic (50 °C) fermentation was found to be superior to the mesophilic one (37 °C), with the biogas production of 354 ml per gram of dry matter, when large-tonnage sewage sludge after filter press (45 % humidity) was fermented using amaranth pulp as the co-substrate. Moreover, in the presence of amaranth pulp, the biomethanogenesis under the mesophilic conditions also increased, the lag phase was almost absent, and the CH_4 level throughout the experiment was about 60 %. As a result, the specific biogas yield reached 251.9 ml per gram of dry matter that is equivalent to ~ 0.25 m^3 of the resultant biogas from 1 kg of organic raw material dry matter. In order to search for the active fraction of amaranth phytomass, we used solvents of different polarity, i.e. dichloromethane, 70 % aqueous ethanol and distilled water. It was found that the lag phase reduced to 10 days with the CH_2Cl_2 and EtOH extracts, which was comparable to that in the presence of dry amaranth phytomass. Obviously, these extracts contain components which either undergo rapid destruction by microorganisms able to turn them into biogas, or contribute to bacterial growth. The dichloromethane extract added to the substrate led to the most efficient biogas production, which is consistent with the literature data. Our findings indicate the ecological and economic feasibility of using amaranth pulp for organic waste bioconversion.

Keywords: *Amaranthus cruentus* L., amaranth, methanogenesis, co-substrate, amaranth phytomass extracts, biogas, sewage sludge, amaranth pulp

Methanic fermentation, or biomethanogenesis, is a known process of biomass-to-energy conversion [1]. Biogas (a mixture of approximately 65% CH_4, 30% CO_2, 1% H_2S and minor amounts of N_2, O_2, H_2, CO) is formed in anaerobic environment, by multielement microbial consortium, capable of converting almost all classes of organic compounds, household, agricultural and some industrial waste into biogas [2]. Methanogenesis is executed by utterly specialised prokaryotes, being very old archebacteria, or archea from the *Methanobacterium*, *Methanosaeta* (*Methanothrix*), *Methanococcus*, *Methanosarcina*, *Methanocorpusculum*, *Methanobrevibacteria* and *Methanopyrus* genera [3-6].

The review of existing works related to obtaining of biogas [7-10] has proved the problem on intensifying of methane fermentation of organic raw material is still urgent. One of the most promising trends consists in search for plant simulators and inhibitors of methanogenesis. E.g., a comparison was made between the composition of volatile fatty acids (VFA) and the output of biogas under the effect of 13 plant extracts, selected according to the highest flavonoid activity [11] (by fermentation of 50:50 mixture of herbal phytomass extract and barleycorn). Experiments found that extracts of *Lavandula officinalis* and *Solidago virgaurea* stimulate fermentation, while those of *Equisetum arvense* and *Salvia officinalis* inhibit the yield of methane. Of special interest is the work that deals with investigation into effect the herbaceous plant extracts (21 species) have on methanogenesis, gram-positive and gram-negative bacteria, their antimicrobial potential, and destruction of dry substance in vitro [12]. The extracts were obtained through the use of methanol, acetone or water, and the content of total sugars, tannins and saponines was determined. Antimicrobial potential was estimated at gram-positive streptococci and staphylococci and gram-negative bacteria *Esherichia coli* and *Enterobacter*. Acetone and methanol extracts of *Eucalyptus globulus* and water extract of *Sapindus mukorossi* and *E. globulus* inhibit methanogenesis in vitro. The study the effect that saponine-rich extracts of *Carduus, Sesbania* and *Knautia* leaves, and of common fenugreek seeds (*Trigonella foenum-graecum*) have on fermentation in the scar, the output of methane and the microbial community [13] has proved that saponines have antiprotozoal activity which is why they do not suppress methanogenesis. The saponines of common fenugreek seeds boost the activity of the scar content and affect the microbial community by reinforcing growth of fiber decayers and suppressing the growing population of fungi.

Various plant biomass added in sediments of treatment facilities has gained wide-spread acceptance in increasing of methane tanks capacity [14]. Added co-substrates, e.g. manure, enrich carbon-poor substrate with an organic substance. Europe employs plants in producing of more than 50% of its biogas [15, 16]. Still urgent is the search of co-substrates for effective bioconversion of organic waste from agriculture and municipal waters into high-methane biogas.

We were the first in studying phytomass and pulp of cultivated amaranth, obtained after extraction of all practically valuable substances, as co-substrates, and in finding of its optimum proportion with substrate (sediment of municipal waters) to enhance formation of biogas and methane fermentation.

The work assessed the effect the red amaranth-based additives have on effective conversion of sedimentary sludge in treatment facilities and of organic waste into high-methane biogas.

Techniques. As a substrate, we used sediments of municipal waters (SMW, humidity 80.4%; compacted SMW, humidity 98.4%; SMW after press-filter, humidity 45.0%) of the city of Kazan, as co-substrates, phytomass, pulp after comprehensive processing (extraction of pectines, rutin and plant protein), and alcohol, dichlormethane and water extracts of pulp of amaranth (*Amaranthus cruentus* L.) plants (Dyuimovochka variety). To find elemental composition (C, H, N and S) of substrate and co-substrate, we employed an analyzer CHN-3 (Khimavtomatika, Russia); streptocide was a standard (C — 41.85 %, H — 4.65 %, N — 16.26 %, S — 18.58 %).

Experiments varied type of substrate, type of co-substrate, substrate-co-substrate ratio, temperature of incubation. Fermentation was made in laboratory reactors that consists of water bath LB-160 and immersion circulating thermostat LT-100 (bottles made of MTO БК3-50 glass, V = 500 ml) (LOIP, Russia).

To compare methane formation depending on co-substrate fraction mixed with SMW (24, 52, 74 and 87 % on absolute dry weight, calculated with regard to humidity of SMW and co-substrate), we used dried phytomass of amaranth; to all sample we added 100.5 g of compacted SMW and fermented at 37 °C. The effect of mesophilic (37 °C) and thermophilic (50 °C) modes of incubation was researched during fermentation of SMW after the filter-press (50.0 g substrate with 100 ml of distilled water added). The same experiment studied the effect the amaranth pulp, as a co-substrate, has on effectiveness of mesophilic (37 °C) fermentation (22.5 g substrate, 16.7 g pulp with 9.2 % humidity; 100 ml of distilled water). To understand the effect of pulp extracts, 0.2 g of dichloromethane (CH_2Cl_2), 0.2 g of alcohol (EtOH) or 2.3.g of amaranth water extract (obtained by evaporation to dryness in rotary evaporator IR-1LT, Russia) was added to substrate (mixture of 37.5 g of SMW and 105.0 g of compacted SMW); for comparison, extracts were replaced with amaranth pulp (5.0 g) (incubation at 37 °C).

Daily volume measurements estimated the output of biogas [17]. Gas-liquid chromatography (GLC) controlled the methane content in samples (CHROM-5, Laboratorní přístroje, Czechia, column 2.4 m with Porapak Q filler, 80-100 mesh, Sigma-Aldrich Co., USA; 80 °C; heat conduction detector, carrier gas helium).

Changes in microorganisms composition in the process of SMW fermentation have been revealed by culture on methanogenic media [18] and Gram staining with further microscopy (MBI-15, LOMO, Russia).

Origin 6.1 siftware was used to process data (https://softadvice.informer.com/Origin_6.1_Free_Download.html). Tables and figures show means (*M*) and standard errors of means (±SEM). Significance of differences was estimated by Student's *t*-criterion. The distinctions were considered statistically significant at p = 0.05.

Results. *Amaranthus cruentus* was chosen because protein-high biomass [19-21] of this high-yielding crop can be an industrially reproducible plant raw material. As a part or research, we developed unique methods and scheme for obtaining rutin, plant protein and pectin during comprehensive processing of amaranth [20] based on extraction from dried phytomass in a single technological cycle.

1. Elemental composition of sediment of municipal waters (SMW), phytomass and amaranth (*Amaranthus cruentus* L., Dyuimovochka variety) pulp used for laboratory simulation of the biogas production

Substrate, co-substrate	C, %	H, %	N, %	C/N
Compacted SMW	34.65	6.20	7.15	4.9
SMW	39.82	7.05	5.81	6.9
Amaranth phytomass	36.98	4.67	3.47	10.7
Amaranth pulp	42.11	6.42	5.20	8.1

Tables 1 and 2 contain properties of substrates and co-substrates used in the experiment. Methanogenesis was simulated in laboratory, with the use of bioreactor (Fig. 1).

2. Properties of substrates having various content of dried amaranth (*Amaranthus cruentus* L., Dyuimovochka variety) phytomass used for laboratory simulation of the biogas production (*n* = 3, *M*±SEM)

Amaranth, %	Dry substance, g	Organic dry substance, g	Humidity, %
24	11.2±0.34	7.3±0.22	92.1±2.76
52	15.9±0.48	11.3±0.34	89.0±2.67
74	18.1±0.54	13.7±0.41	86.7±2.60
87	21.5±0.65	16.9±0.51	83.2±2.50

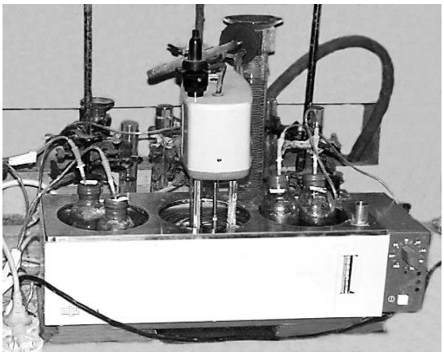

Fig. 1. Laboratory facility for biogas obtaining (V = 500 ml, LOIP, Russia).

Kinetics of CH_4 formation with 24, 52, 74 and 87 % of dried amaranth phytomass added as co-substrate (Fig. 2) has proved the biogas yield is the highest at 24% (291.1 ml/g of dry substance, $p = 0.05$). The effect retained for 50 days (Table 3), with approximately 60% of CH_4 in biogas (see Fig. 2). More objective criterion of the process efficiency is a specific yield of biogas in terms of content of organic dry substance in the substrate that, too, was high (see Table 3).

Adding of 52% of amaranth produced 226.6 ml/g of biogas in dry substance (true at $p = 0.05$). Biogas had been forming for 138 days and contained, approximately, 55% of methane (see Fig. 2).

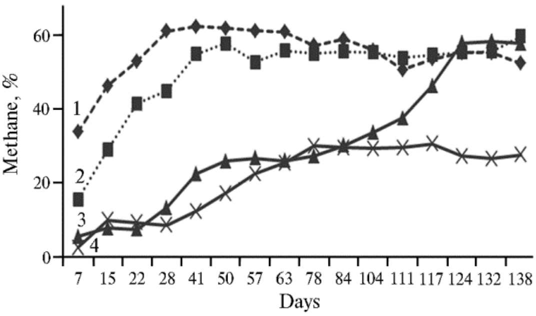

Fig. 2. Kinetics of CH_4 formation during bioconversion of sediment of municipal waters vs. content of amaranth (*Amaranthus cruentus* L., Dyuimovochka variety) dry biomass in the substrate: 1 — 24 %, 2 — 52 %, 3 — 74 %, 4 — 87 % (average for 3 repetitions, laboratory trial).

With 74% amaranth phytomass content, the process had prolonged lag phase: gas formation activated no sooner than in 110 days. Excess of amaranth suppressed methanogenesis, and specific output of biogas made 127.8 ml/g in dry substance (see Table 3). With 87% amaranth, biogas contained not more than 30% of methane (see Fig. 2) at biogas yield of 29.1 ml/g in dry substance ($p = 0.05$, see Table 3).

3. Production of biogas during bioconversion of sediment of municipal waters vs. content of amaranth (*Amaranthus cruentus* L., Dyuimovochka variety) dry phytomass in substrate (*n* = 3, *M*±SEM, true at 5% significance level, laboratory trial)

Amaranth, %	Specific yield		
	ml gas/ml substrate	ml gas/gdry substance	ml gas/g organic dry substance
24	23.0±1.15[a]	291.1±14.60	445.1±22.26
52	24.9±1.25[a]	226.6±11.33	318.5±15.93
74	17.0±0.85	127.8±6.39	168.4±8.42
87	4.7±0.24	29.1±1.46	36.9±1.85

Note. Between variants marked as [a] there are no statistically significant differences at $p = 0.05$.

A comparison of these results with the data presented in the Table 2 has proved optimum humidity of substrate for biogas should be not less than 90%, and gas formation decreases with its reduction. Consequently, adding of amaranth phytomass to substrate was only effective at certain quantitative relationship with SMW, whereas excessive amaranth phytomass suppressed methanogenesis.

Figure 3 illustrates dynamics of SMW pH change with 52% amaranth phytomass added.

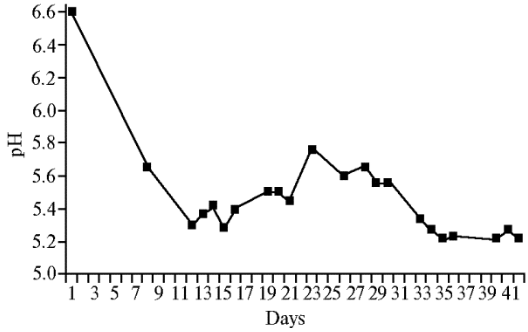

Fig. 3. Rise of acidity during anaerobic fermentation of sediment of municipal waters with 52% dry biomass of amaranth (*Amaranthus cruentus* L., Dyuimovochka variety) (average for 3 repetitions, laboratory trial).

Lag phase in mesophilic mode took 30 days, when co-substrate was amaranth pulp, remained after comprehensive processing and extraction of pectines, rutin and plant protein [22, 23]. On obtaining of maximum daily yield of biogas (appr. 120 ml on day the 40), high content (60%) of CH_4 was noted in it (Fig. 4). Specific productivity in experiment made 134.7 ml/g of dry substance (p = 0.05, Table 4). In thermophilic mode, the volume of the gas formed was considerably greater: for longer than 20 days, its yield exceeded 200 ml. In addition, lag phase reduced to 14 days when biogas contained more than 50% of CH_4 (see Fig. 4).

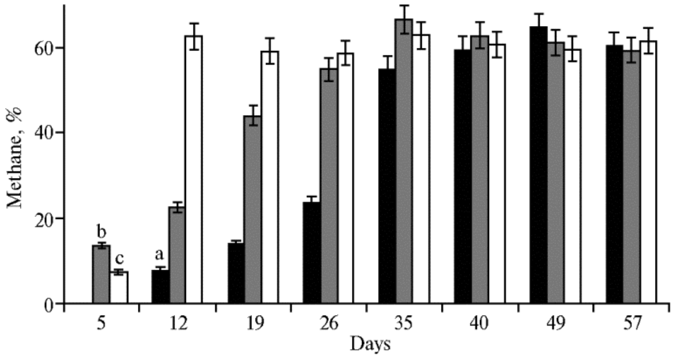

Fig. 4. Content of CH_4 in biogas at various temperature modes for anaerobic fermentation of sediment of municipal waters (SMW) **in the presence of amaranth (*Amaranthus cruentus* L., Dyuimovocka variety) pulp:** a — SMW at mesophilic mode (37 °C), b — SMW at thermophilic mode (50 °C), c — SMW, and pulp at mesophilic mode (*n* = 3, p = 0.05, laboratory trial).

4. Biogas production during bioconversion of sediment of municipal waters (SMW) **vs. temperature mode and content of amaranth (*Amaranthus cruentus* L., Dyuimovochka variety) pulp in substrate** (*n* = 3, *M*±SEM, true at 5% significance level, laboratory trial)

Composition	Mode	Specific yield	
		ml gas/ml substrate	ml gas/g dry substance)
SMW	Mesophilic, 37 °C	20.5±1.03^a	134.7±6.74
SMW + amaranth pulp	Mesophilic, 37 °C	22.7±1.14^a	251.9±12.60
SMW	Thermophilic, 50 °C	53.1±2.66	354.0±17.70

Note. Between variants marked as ^{a} there are no statistically significant differences at p = 0.05.

Specific yield of biogas made 354.0 ml/g of dry substance (p = 0.05), that points to the advantage of thermophilic mode of SMW amaranth-free fermentation. Amaranth added to substrate increased yield of biogas in mesophilic mode (with lag phase almost omitted, on day 12 the volume of biogas reached 80 ml for 62% of CH_4) (see Fig. 4). It was only after 60 days, that yield of gas dropped to 20 ml, with approximately 60% of CH_4, before the experiment had been completed. With amaranth pulp added in mesophilic mode, specific yield of biogas made ~ 0.25 m^3 per 1 kg of dry substance of organic raw material.

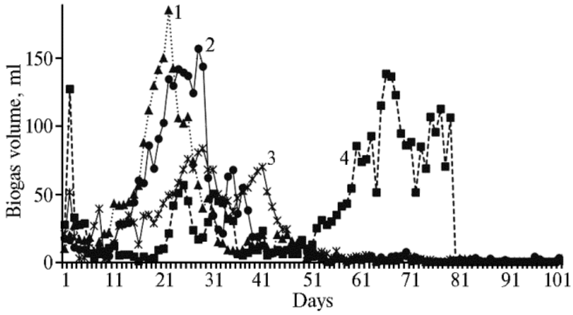

Fig. 5. Kinetics of biogas production during bioconversion of sediment of municipal waters with added alcohol (1), **dichlormethane** (2) **and water** (3) **extracts and amaranth (*Amaranthus cruentus* L., Dyuimovocka variety) pulp** (4) (average for 3 repetitions, laboratory trial).

Dichlormethane extract of amaranth phytomass [22] has green color through the presence of chlorophyll, water extract contains carbohydrates, proteins, mineral salts and amarantite, whereas the ethanol extract is rich in phenol compounds rutin and quercetin. After three extractions, the pulp contained fiber, carbohydrates and pectines [22]. Kinetics of gas formation observed during mesophilic fermentation of SMW and compacted SMW mixture (Fig. 5) has proved that CH_2Cl_2 and EtOH extracts added to substrate reduced lag phase to 10 days. This effect is comparable with that of amaranth phytomass. Clearly, dichlormethane and ethanol extracts contain components that either destruct easily when exposed to microorganism community converting them to biogas, or help in the growth of biomass. CH_2Cl_2 extract added to substrate marked the most effective formation of biogas, which is consistent with the literature data [11, 24-27] (Table 5).

5. Production of biogas during bioconversion of sediment of municipal waters with various extracts and amaranth pulp (*Amaranthus cruentus* L., Dyuimovochka variety) added ($n = 3$, $M \pm$SEM, true at 5% significance level, laboratory trial)

Co-substrate	Specific yield		
	ml gas/ml substrate	ml gas/g dry substance	ml gas/g organic dry substance
CH_2Cl_2 extract	162 ± 0.81^{a}	266.1 ± 13.31^{b}	433.5 ± 21.68
EtOH extract	14.5 ± 0.73^{a}	$236.1 \pm 11.81^{c,\ d}$	382.5 ± 19.13^{e}
Water extract	16.6 ± 0.83^{a}	221.8 ± 11.09^{c}	345.8 ± 17.29^{e}
Amaranth pulp	23.7 ± 1.19	$259.1 \pm 12.96^{b,\ d}$	355.4 ± 17.77^{e}

Note. Between variants marked as ^{a} there are no statistically significant differences at p = 0.05.

With pulp added, specific yield of biogas made 259.1 ml/g of dry substance (p = 0,05), that can be related to a similar result (266.1 ml/g of dry substance) for extract obtained through the use of CH_2Cl_2, with 82.5% of CH_4 on day 98 (maximum value over the whole period of studies). We point out that both amaranth pulp after three extractions (see Table 5) and amaranth phytomass (see Table 3) serve as an activating co-substrate for methanogenesis (RF Patent No. № 2351552). Hence, in the presence of amaranth pulp, biomethanogenesis, too, increases its productivity in mesophilic mode (by 12.8 %, p = 0.05), that, in general, increases the efficiency of comprehensive processing of raw obtained during growing of this plant. The results presented have confirmed environmental and economic feasibility of amaranth pulp use.

Microbiological study of samples during anaerobic fermentation of SMW has proved that typical of the original substrate is the presence of large eukaryotic forms. Gram-positive small rod bacteria, both single ones and in long chains, dominated in the medium at the maximum activity of gas formation (day 40). Very small single forms were more typical for acetate-based nutrient medium. Smooth and rough colonies grew on MPA [18]. Considerable amount of very small colonies has grown at the medium for methanogens. Both gram-positive and gram-negative forms were found.

Eventually, our studies have proved that optimized substrate of organic substance with the use of amaranth phytomass/pulp affords higher effective production of biogas from the sediment of municipal waters, thus attacking the problem of waste disposal and getting the fuel from a cheap renewable source.

REFERENCES

1. Varfolomeev S.D., Efremenko E.N., Krylova L.P. *Uspekhi khimii*, 2010, 79(6): 544-564 (in Russ.).
2. Varfolomeev S.D., Moiseev I.I., Myasoedov B.F. *Vestnik Rossiiskoi akademii nauk,* 2009, 79(7): 595-604 (in Russ.).
3. Gerardi M.H. *The microbiology of anaerobic digesters*. John Wiley & Sons, Inc., Hoboken, New Jersey, 2003.
4. Schiraldi C., Giuliano M., de Rosa M. Perspectives on biotechnological applications of archaea. *Archaea*, 2002, 1(2): 75-86 (doi: 10.1155/2002/436561).
5. Vogt C., Kleinsteuber S. Richnow H.-H. Anaerobic benzene degradation by bacteria. *Microbial Biotechnol.*, 2011, 4(6): 710-724 (doi: 10.1111/j.1751-7915.2011.00260.x).
6. Fowler S.J., Dong X., Sensen C.W., Suflita J.M., Gieg L.M. Methanogenic toluene metabolism: community structure and intermediates. *Environ. Microbiol.*, 2012, 14(3): 754-764 (doi: 10.1111/j.1462-2920.2011.02631.x).
7. Ahring B.K., Ellegaard L., Angelidaki I., Schmidt J.E., Gavala H.N., Skiadas I.V., Ahring B.K., Haagensen F., Dolfing J., Mogensen A.S., Lyberatos G., Stamatelatou K., Pind P.F. *Biomethanation II.* B.K. Ahring (ed.). Springer, Berlin, 2003 (doi: 10.1007/3-540-45838-7).
8. Deublein D., Steinhauser A. *Biogas from waste and renewable resources.* Wiley-VCH Verlag GmbH & Co. KGaAiley, Weinheim, Germany, 2008 (doi: 10.1002/9783527632794).
9. Samer M. Biogas plant constructions. In: *Biogas.* S. Kumar (ed.). InTech, Rijeka, Croatia, 2012: 343-368.
10. Pisarikova B., Peterka J., Trackova M., Moudry J., Zraly Z., Herzig I. Chemical composition of the above-ground biomass of *Amaranthus cruentus* and *A. hypochondriacus. Acta Vet. BRNO*, 2006, 75(1): 133-138.
11. Broudiscou L.P. Papon G., Broudiscou A.F. Effects of dry plant extracts on fermentation and methanogenesis in continuous culture of rumen microbes. *Anim. Feed Sci. Technol.*, 2000, 87(3-4): 263-277 (doi: 10.1016/S0377-8401(00)00193-0).
12. Sirohi S.K., Pandey N., Goel N., Singh B., Mohini M., Pandey P., Chaudhry P.P. Microbial activity and ruminal methanogenesis as affected by plant secondary metabolites in different plant extracts. *International Journal of Environmental Science and Engineering*, 2009, 1(1): 52-58.
13. Goel G., Makkar H.P.S., Becker K. Changes in microbial community structure, methanogenesis and rumen fermentation in response to saponin-rich fractions from different plant materials. *J. Appl. Microbiol.*, 2008, 105(3): 770-777 (doi: 10.1111/j.1365-2672.2008.03818.x).
14. Mursec B., Vindis P., Janzekovic M., Brus M., Cus F. Analysis of different substrates for processing into biogas. *Journal of Achievements in Materials and Manufacturing Engineering*, 2009, 37(2): 652-659.
15. Karpenstein-Machan M. *Energiepflanzenbau für Biogasanlagenbetreiber.* DLG-Verlag, Frankfurt am Main, 2005.
16. Ferry J.G., Kastead K.A. Methanogenesis. In*: Archaea: molecular and cellular biology.* R. Cavicchioli (ed.). ASM Press, Washington, 2007, Ch. 13: 288-314.
17. Guben-Veil' N.N. *Metody organicheskoi khimii* [Methods of organic chemistry]. Moscow, 1963 (in Russ.).
18. Netrusov A.I., Kotova I.B. *Mikrobiologiya* [Microbiology]. Moscow, 2006 (in Russ.).
19. Minzanova S.T., Mironov V.F., Konovalov A.I., Vyshtakalyuk A.B., Tsepaeva O.V., Mindubaev A.Z., Mironova L.G., Zobov V.V. *Pektiny iz netraditsionnykh istochnikov: tekhnologiya, struktura, svoistva i biologicheskaya aktivnost'* [Pectins from non-traditional sources: technology, structure, properties and biological activity]. Kazan', 2011 (in Russ.).
20. Gins V.K., Kononkov P.F., Pivovarov V.F., Gins M.S. [Creation of varieties and hybrids of vegetable crops with increased content of biologically active substances and antioxidants]. *Agricultural Biology*, 2003, 1: 108-113 (in Russ.).
21. Vysochina G.I. *Khimiya rastitel'nogo syr'ya*, 2013, 2: 5-14 (in Russ.).
22. Minzanova S.T., Mironov V.F., Vyshtakalyuk A.B., Tsepaeva O.V., Mironova L.G., Konovalov A.I. *Izvestiya AN. Ser. khim.*, 2014, 9: 2142-2155 (in Russ.).
23. Mindubaev A.Z., Minzanova S.T., Skvortsov E.V., Mironov V.F., Zobov V.V., Akhmadullina F.Yu., Mironova L.G., Belostotskii D.E., Konovalov A.I. *Vestnik Kazanskogo tekhnologicheskogo universiteta,* 2009, 4: 220-226 (in Russ.).
24. Oh S., Shintani R., Koike S., Kobayashi Y. Ginkgo fruit extract as an additive to modify rumen microbiota and fermentation and to mitigate methane production. *J. Dairy Sci.*, 2017, 100(3): 1923-1934 (doi: 10.3168/jds.2016-11928).

25. Perna F.F. Jr., Cassiano_E.C.O., Martins_M.F., Romero_L.A., Zapata_D.C.V., Pinedo_L.A., Marino_C.T., Rodrigues P.H.M. Effect of tannins-rich extract from *Acacia mearnsii* or monensin as feed additives on ruminal fermentation efficiency in cattle. *Livest. Sci.*, 2017, 203: 21-29 **(**doi: 10.1016/j.livsci.2017.06.009**).**
26. Medjekal S., Bodas R., Bousseboua H., Lypez S. Evaluation of three medicinal plants for methane production potential, fiber digestion and rumen fermentation in vitro. *Energy Procedia*, 2017, 119: 632-641 (doi: 10.1016/j.egypro.2017.07.089).
27. Bodas R., Prieto N., García-González R., Andrés S., Lypez S. Manipulation of rumen fermentation and methane production with plant secondary metabolites. *Anim. Feed Sci. Tech.*, 2012, 176(1-4): 78-93 (doi: 10.1016/j.anifeedsci.2012.07.010).