Agronomy Research **15**(3), 639–648, 2017

Fungicidal activity of ultradisperse humic sapropel suspensions

N.V. Barakova[1,*], N.Y. Sharova[1,2], A.R. Juskauskajte[2], A.S. Mityukov[3], V.A. Romanov[1] and D. Nsengumuremyi[1]

[1]Saint Petersburg National Research University of Information Technology, Mechanics and Optics (ITMO University), Department of Food Biotechnology and Engineering, Food Biotechnology (Vegetable Stock) Research Body, 9 Lomonosova st., RU191002 Saint Petersburg, Russia
[2]Federal State-funded Scientific Establishment 'All–Russia Research Institute for Food Additives' (GBNU VNIIPD), Department of Food Microingredients Processing, 55 Liteyniy ave., RU191014 Saint Petersburg, Russia
[3]Federal State-funded Scientific Establishment 'RAS Lake Ecology Institute' (INOZ RAN), 9 Syevast'yanova st., RU196105 Saint Petersburg, Russia
[*]Correspondence: n.barakova@mail.ru

Abstract. The study compared the effect of two ultradisperse humic sapropel suspensions (UDHSS), obtained in Seryodka deposit (Pskov region, Russia), on viability of *Aspergillus niger* in four experiments. In Experiment 1, *Aspergillus niger* strain L–4 conidia with titre (3.1–3.7) × 10^3 CFU cm^{-3} were suspended in 0.1 cm^3, 1.0 cm^3, 2.0 cm^3, 3.0 cm^3, or 5.0 cm^3 of either UDHSS, and immediately incubated on wort agar. In Experiments 2 and 3, *Aspergillus niger* L–4 conidia with the same titre were suspended in and remained in contact with 0.1 cm^3, 1.0 cm^3, 2.0 cm^3, 3.0 cm^3, or 5.0 cm^3 of UDHSS for 2 or 24 hours at (20.5 ± 0.5) °C (68.9 ± 0.9°F) and incubated on wort agar. The number of colonies in Petri dishes upon 24 hours and 5 days of cultivation was observed. In Experiment 4, contamination of barley grain and distillers' dry grain (DDG) was simulated. DDG treated with 20 cm^3 kg^{-1} of either suspension was inseminated with *Aspergillus niger* L–4 at (5.1–5.3) × 10^3 CFU cm^{-3}, and put under standard storage conditions, changes of microflora examined at 24 hour intervals during 5 days. As a result of Experiment 1, in consideration to statistical significance, there was little fungicidal action on *Aspergillus* conidia. In Experiments 2 and 3, a prominent fungicidal effect was demonstrated by both sapropel suspensions. In Experiment 4, both types of suspensions exhibited a statistically significant effect on *Aspergillus* conidia only in samples previously remaining in contact with 3.0 or 5.0 cm^3 of suspension. Amplitude of the effect proved to be dependent on both UDHSS dosage and time of contact (2 or 24 hours).

Key words: *Aspergillus niger*, distillers' dry grain, ultradisperse sapropel suspensions, fungicidal activity.

INTRODUCTION

Sapropel is benthos found in bodies of still fresh water, formed under anaerobic conditions from dead organic matter of hydrobiotic microflora and microfauna. The principal components of sapropels are minerals and organic compounds known as humic substances. The ratio between mineral and organic components would change depending

on depth of sapropel deposition, size of the body of water, its natural surroundings, and many other factors (Avdeyeva et al., 2009).

Humic substances in sapropels contain three types of compounds: fulvic acids, humic acids and humin (Shtin, 2005; Kosov, 2007; Buzlama et al., 2010).

Fulvic acids are components of humus comprising a pronounced peripheral area and a less prominent aromatic core. Fulvic acids are the first products of humification (Shtin, 2005).

Humic acids are components of humus comprising a non-pronounced peripheral area and a prominent heterocyclic core. Hydrogen atoms in prosthetic groups are easily substituted by metal ions, forming salts (humates) (Kireycheva, 2006).

Humin is a non-dissolvable component of humus containing both fulvic acid and humic acid residues strongly bonded with mineral soil components. Such complexes are also known as ulmins (Shtin, 2005; Lishtvan et al., 2012).

The minerals contained in sapropels are non-metallic ions of carbon, phosphorus, silicon, and sulphur, and multiple trace elements: beryllium, boron, brome, chrome, among others.

Previously, several studies have suggested that some of said components may cause antimicrobial activity of sapropels (Kireycheva & Khokhlova, 2000; Gorbunovskaya & Kurzo, 2001; Dolgopolov, 2006; Kulikova et al., 2013; Platonov et al., 2014).

Substandard procedures in storage of food industry produce and raw materials may cause contamination by a microflora of bacteria and fungi. Contamination not only compromises food safety, but may also cause loss of valuable nutrients, since very active hydrolytic enzymes can be synthesized by some of the microorganisms causing spoilage, including *Aspergillus niger* fungus.

Due to high activity of its hydrolases (mainly amylases), *Aspergillus niger* is used for obtaining commercial enzymes. High output citric and gluconic acid production from molasses or a starchy material, e.g. grain, can also be achieved with specialized strains of the fungus (Vybornova & Sharova, 2015).

To prevent contamination and deterioration of stored food and raw materials, many physical and chemical means can be applied (Abraskova et al., 2013). In biotechnological practices, where sterility is vital, various antiseptics (Sharova et al., 2012) and other means of antimicrobial action (Sharova & Kamen'kova, 2012) are also used.

The studies mentioned above (Gorbunovskaya & Kurzo, 2001; Dolgopolov, 2006; Kulikova et al., 2013; Platonov et al., 2014) provide proof that humic substances and particularly humates contain microbial growth, the amplitude of the effect being on par with synthetic antibiotics such as hydrocortisone. Inhibitory effect towards *Staphylococcus aureus*, *Escherichia coli* and other bacteria, as well as *Candida* yeast, was demonstrated. To make use of biologic activity of humic salts, sapropels should be subjected to extraction. It is believed that variations in extraction techniques affect properties exhibited by the recovered material, as more bioactive substances may be made available with more rigorous treatment (Gostischeva et al., 2004).

The containing effect may prove useful in pharmacology and food industry, including food production facilities that recycle other manufacturers' by-products. One such by-product is distillers' dry grain (DDG), which can be used as feed at livestock plants. Adding antibiotics to DDG has been an accepted practice for years, but is ceding (Jacques, 2003) due to growing awareness of antibiotic misuse danger for both humans

and animals (World Health Organization, 2015). Sapropel treatment of animal feed reportedly improves livestock productivity (Kireycheva & Khokhlova, 2000; Dolgopolov, 2006), which provides an alternative to antibiotics in feed preservation.

The present study aimed to demonstrate potential of sapropels as anti-spoilage agents for grain and DDG storage stability assurance.

MATERIALS AND METHODS

The objects of the study were ultradisperse humic sapropel suspensions (referred to from here on as 'UDHSS–1' and 'UDHSS–2') obtained in RAS Lake Ecology Institute with alkaline extraction and ultrasound treatment of air-dry sapropels from Seryodka deposit (Pskov region, Russia). Both samples were subjected to ultrasound at 35 kHz frequency and pressure of 2 W cm^{-2}. The principal difference between the two suspensions was more intense thermal treatment during UDHSS–2 extraction: 40 °C (104 °F) as compared to 20 °C (68°F) for UDHSS–1.

The materials of the study were barley of 2015 autumn harvest, as well as distillers' dry grain (DDG). DDG was recovered from whole stillage as 'wet cake' at 30% moisture and dehydrated to 10% moisture to imitate standard storage humidity.

Automatic titrator 848 Titrino Plus (Metrohm) was used for determination of UDHSS pH. The device operates in two modes: titration with automatic endpoint determination and pH measurement, the latter used in the experiments (other preferences by default). Time of assay was no less than 3 minutes.

Refractometer PTR 46 (Index Instruments) was used for assessment of UDHSS extract, under the manufacturers protocol 'Brix measurement with temperature correction'.

Moisture analyzer MOC120H (Shimadzu) was used for assessment of all materials moisture content, under the manufacturer's protocol 'Slow drying method' with automatic endpoint determination. The endpoint was determined as the point at which mass drift fell below the preset value of 0.001 g.

Polarimeter PolAA FF55 (Optical Activity) was used for measuring apparent starch in barley and DDG according to Ewers method (ISO/TC 93, 1997).

Protein content in DDG was measured according to ASBC method Brewers' Grains–7 (ASBC, 1992).

Humic acid content was measured with ISO method 19822:89 (EC Joint Research Centre, 1989).

Chromium, copper, iron, manganese, and zinc content was measured according to ISO method 17321:2008 (EC Joint Research Centre, 2008).

Cadmium and lead content was measured with ISO method 17318:2015 (EC Joint Research Centre, 2015).

Lipid content in DDG was measured with ISO method 3596:2000 (ISO/TC 34, 2000).

The effect of UDHSS on vitality of *Aspergillus niger*, a common contaminant of stored grain, as well as stored DDG, was studied in four experiments. Strain L–4 of *Aspergillus niger*, chosen for its high enzymatic activity, was cultivated in 750 cm^3 shaker flasks on the following medium: 150.00 g dm^{-3} carbon source; 2.50 g dm^{-3} ammonium nitrate (NH_4NO_3); 0.25 g dm^{-3} heptahydrous magnesium sulphate ($MgSO_4$

$7H_2O$); 0.16 g dm^{-3} potassium phosphate (KH_2PO_4); water enough to get 750 cm^3; pH 6.5 (Mushnikova et al., 2001; Vybornova & Sharova, 2015).

In order to assess antifungal activity of UDHSS, their respective minimum inhibitory concentrations were determined with the common dilution method (EUCAST, 2017). Broth microdilution is usually advised, but for single-strain cultures, 1 cm^3 dilution (macrodilution) may be used, as fidelity loss is negligible due to strain uniformity of the culture. Additionally, wort agar was used instead of broth so that all samples could be kept together in the same incubators. Total number of colony-forming units (CFU) in 1 cm^3 suspensions after incubation on wort agar at 32 or 37 °C (90 or 100°F) was calculated. 1 cm^3 of each suspension was mixed with 8–12 cm^3 wort agar at 45 °C (113°F), before cooling of the agar, in a sterile Petri dish, two samples per each suspension, and incubated one at 32 °C (90°F) and the other at 37 °C (100°F) for (24 ± 2) hours stacked upside down.

In Experiment 1, *Aspergillus niger* strain L–4 conidia with titre (3.1–3.7) × 10^3 CFU cm^{-3} were suspended in either UDHSS, the volume of the suspensions varying between 0.1 cm^3, 1.0 cm^3, 2.0 cm^3, and 5.0 cm^3. Immediately after, the conidia were incubated on wort agar.

In Experiments 2 and 3, *Aspergillus niger* L–4 conidia with the same titre were suspended and remained in contact with in 0.1 cm^3, 1.0 cm^3, 2.0 cm^3, or 5.0 cm^3 of one of UDHSS for 2 or 24 hours at (20.5 ± 0.5) °C (68.9 ± 0.9°F) and incubated on wort agar. The number of colonies in Petri dishes upon 24 hours of cultivation was observed for all suspended *Aspergillus* samples.

In Experiment 4, contamination of barley grain and distillers' dry grain (DDG) was simulated. Grains of barley used in the study were sampled at a warehouse and proved to be previously contaminated with spoilage microorganisms (but not *Aspergillus niger*) which made the experiment resemble actual storage conditions. Barley and DDG were treated with 20 cm^3 of either sapropel suspension per 1 kg. The materials were then inseminated with *Aspergillus niger* L–4 at (5.1–5.3) × 10^3 CFU cm^{-3}, put under standard storage conditions, and examined at for changes of microflora 24 hour intervals during 5 days.

RESULTS AND DISCUSSION

Physical and chemical composition of UDHSS, barley, and DDG

According to UDHSS assay carried out, the suspensions were equally strong alkali but were quite different in almost every other aspect. UDHSS–2 had extract, sugars, lipids, and many metals content, especially iron, higher compared to UDHSS–1 (Table 1).

Basic physical and chemical properties of barley grain were assayed (Table 2) and found to comply with food grade standards for moisture content, Ewers apparent starch, and foreign particles content (Skurikhin & Tutel'ian, 2002).

Table 1. Physical and chemical properties of UDHSS samples

Parameter	Measurement unit	Value	
		UDHSS-1	UDHSS-2
pH		13.10	13.20
Extract	°Plato	4.61	14.95
Sugars	mg cm^{-3}	3.20	5.50
Lipids	mg cm^{-3}	275.00	1,070.00
Humic acids	% (dry matter)	20.70	38.72
Trace metals	mg kg^{-1}		
Cd		1.35	1.34
Co		7.49	7.09
Cr		1.23	4.00
Cu		0.96	9.15
Fe		8.28	456.3
Mn		4.46	4.34
Ni		6.92	6.78
Pb		8.11	9.62
Zn		2.38	1.94

Table 2. Physical, chemical and nutritive properties of barley grain

Parameter	Measurement unit	Value
Moisture	%	8.51 ± 0.10
Apparent starch	% (dry matter)	56.70 ± 1.10
Foreign particles		0.69 ± 0.10

Physical, chemical and nutritive properties of DDG were also assayed (Table 3).

Table 3. Physical, chemical and nutritive properties of distillers' dry grain

Parameter	Measurement unit	Value
pH	–	4.67
Extract	°Plato	90.60
Sugars	% (dry matter)	12.70
Crude protein	% (dry matter)	27.10 ± 1.90
Digestible protein	% dry (matter)	21.10 ± 1.10
Hydrolysable carbohydrates	% (dry matter)	2.25 ± 0.11
Total lipid	% (dry matter)	5.40 ± 0.30
Total ash	% (dry matter)	4.49 ± 0.22
Total fibre	% (dry matter)	7.00 ± 0.40

Experiments 1–3. **Fungicidal effect of UDHSS on *Aspergillus niger* conidia**

In the first three experiments, fungicidal effect of UDHSS on *Aspergillus* conidia was assayed after 24 hours of incubation on wort agar (Table 4).

Table 4. Fungicidal effect of UDHSS after 24 hours of incubation

Sample name	Time at (20.5 ± 0.5) °*C* (69 °*F*), hours	Volume of UDHSS, cm^3				
		0.1	1.0	2.0	3.0	5.0
Fungal growth in Experiment 1, *CFU* cm^{-3} ($P < 0.05$)						
UDHSS–1	–	$3–4 \times 10^3$	$3–4 \times 10^3$	$3–4 \times 10^3$	50–200	50–200
UDHSS–2	–	$3–4 \times 10^3$	$3–4 \times 10^3$	$3–4 \times 10^3$	–	5–10
Fungal growth in Experiments 2 and 3, *CFU* cm^{-3} ($P < 0.01$)						
UDHSS–1	2	5–10	5–10	–	–	–
UDHSS–2	2	5–10	5–10	–	–	–
UDHSS–1	24	–	–	–	–	–
UDHSS–2	24	–	–	–	–	–

In Experiment 1, fungicidal effect of UDHSS on *Aspergillus* conidia was observed in samples with 3.0 or 5.0 cm^3 of a suspension only. UDHSS–2 has exhibited a stronger fungicidal effect than UDHSS–1.

In Experiments 2 and 3, both types of suspensions had a significant fungicidal effect on *Aspergillus* conidia only in samples previously remaining in contact with 3.0 or 5.0 cm^3 of UDHSS, more notably UDHSS–2.

Additionally, fungicidal effect on *Aspergillus* conidia upon 5 days of incubation was assayed (Table 5).

Table 5. Fungicidal effect of UDHSS after 5 days of incubation

Sample name	Time at (20.5±0.5) °C (68.9 °F), hours	Volume of UDHSS, cm^3				
		0.1	1.0	2.0	3.0	5.0
Fungal growth in Experiment 1, CFU cm^{-3} ($P < 0.05$)						
UDHSS–1	–	$3–4 \times 10^3$	$3–4 \times 10^3$	$3–4 \times 10^3$	$3–4 \times 10^3$	$3–4 \times 10^3$
UDHSS–2	–	$3–4 \times 10^3$	$3–4 \times 10^3$	$3–4 \times 10^3$	$3–4 \times 10^3$	$3–4 \times 10^3$
Fungal growth in Experiments 2 and 3, CFU cm^{-3} ($P < 0.01$)						
UDHSS–1	2	$3–4 \times 10^2$	$3–4 \times 10^2$	5–10	–	–
UDHSS–2	2	$3–4 \times 10^2$	$3–4 \times 10^2$	5–10	–	–
UDHSS–1	24	50–200	50–200	5–10	–	–
UDHSS–2	24	50–200	50–200	5–10	–	–

During 5 days of incubation, again both types of suspensions had a notable effect on *Aspergillus* conidia only if contact with 3.0 or 5.0 cm^3 of a suspension was maintained.

Experiment 4. Effect of sapropel extracts on barley grain and DDG spoilage microflora

Grains of barley used in the study proved to be previously contaminated with spoilage microorganisms, but not *Aspergillus niger* (Table 6).

UDHSS had an effect on spoilage microflora, including *Aspergillus niger*, in all samples, but it was only in samples with 0.5 cm^3 or more UDHSS that a lasting antimicrobial effect was observed.

Table 6. The effect of UDHSS on barley grain spoilage microflora

Sample name	Volume of UDHSS, cm^3	Time of storage, hours				
		24	48	72	96	120
Control (no *Aspergillus*)	–	‡	‡	‡‡	‡‡	‡‡‡
Control (with *Aspergillus*)	–	‡ 50–200	‡ 50–200	‡‡ 50–200	‡‡ 50–200	‡‡ $3–4 \times 10^2$
UDHSS–1	0.1	–	+	+	‡ 5–10	‡ 5–10
UDHSS–2	0.1	–	–	–	‡ 5–10	‡ 5–10
UDHSS–1	0.5	–	–	–	5–10	‡ 5–10
UDHSS–2	0.5	–	–	–	–	5–10
UDHSS–1	1.0	–	–	–	–	–
UDHSS–2	1.0	–	–	–	–	–

Legend. «‡»: growth density of other microorganisms than Aspergillus niger. «–»: no growth; «‡»: moderate growth; «‡‡»: significant growth; «‡‡‡»: dense growth.

DDG used in the study proved to be previously contaminated with spoilage microorganisms., but not *Aspergillus niger* (Table 7).

Table 7. The effect of UDHSS on DDG spoilage microflora

Sample name	Volume of UDHSS, cm^3	Time of storage, hours				
		24	48	72	96	120
Control (no *Aspergillus*)	–	–	–	‡‡	‡‡	‡‡‡
Control (with *Aspergillus*)	–	–	‡ 50–200	‡ 50–200	‡ 50–200	‡‡ $3–4 \times 10^2$
UDHSS–1	0.1	–	–	–	–	+
UDHSS–2	0.1	–	–	–	–	–
UDHSS–1	0.5	–	–	–	–	–
UDHSS–2	0.5	–	–	–	–	–
UDHSS–1	1.0	–	–	–	–	–
UDHSS–2	1.0	–	–	–	–	–

Legend. «‡»: growth of other microorganisms than Aspergillus niger. «–»: no growth; «‡»: moderate growth; «‡‡»: significant growth; «‡‡‡»: dense growth.

After 5 days of incubation, both types of suspensions have shown a significant fungicidal effect on *Aspergillus* conidia, more notably UDHSS–2. While amplitude of the effect again proved to be proportional to suspension volume, the dosage necessary for an antimicrobial effect to show was 6 times less, 0.5 cm^3, compared to the effective minimum in the previous experiments, 3.0 cm^3 UDHSS. This can be attributed to some constituents of barley kernels and DDG that may exhibit a synergic antimicrobial effect if the stored material is treated with UDHSS.

CONCLUSIONS

In the current study, ultradisperse humic sapropel suspensions have proven to have significant fungicidal properties, consistently dependent on volume applied, even in small doses. UDHSS–2 sample was found to exhibit higher antifungal potency, probably due to more fungicidal components extracted with more intense thermal treatment.

The pronounced containing effect of sapropel suspensions on *Aspergillus niger* conidia may be attributed to inhibition of *Aspergillus* cell growth and enzyme synthesis by copper, iron and zinc atoms. Since UDHSS–2 contained ten times more copper and 55 times more iron compared to UDHSS–1, a conclusion can be drawn that these metals are at least partly responsible for loss of fungal viability.

Additionally, lipids contained in both suspensions may affect spore surface permeability, preventing nutrient uptake and spore germination.

As an adverse effect, some amounts of nutrients and vitamins contained in the suspensions may cause development of microbial colonies other than *Aspergillus niger*. Careful dosage of UDHSS for antimicrobial use is therefore advised.

Fungicidal properties of UDHSS may find application in industrial grain, and by-products such as distillers' grain, storage procedures. Sapropel can be found in lakes of various regions, so its use for economical hygienic procedures in food and microbial biotechnology is open to consideration. However, properties of sapropels may vary significantly, which requires study of its chemical composition for extraction techniques, suspension dosage and exposure time adjustment.

REFERENCES

Abraskova, S.V., Shashko, Y.K. & Shashko, M.N. 2013. Biological Safety of Feedstuffs. Minsk, 256 p. (in Russian).

ASBC (American Society of Brewer Chemists). 1992. Brewers' Grains–7. Protein (N × .25) by Combustion. Available: http://methods.asbcnet.org/methods/BrewersGrains–7.pdf. Addressed 03.31.2017.

Avdeyeva, L.N., Kovalyenko, T.A. Krivonos, O.I., Plaksin, G.V. & Strunina, N.N. 2009. Determination of sapropel chemical composition // *Chemistry and Chem*. *Eng*. **52**(3), 121–123 (in Russian).

Buzlama, A.V. & Chernov, Y.N. 2010. Pharmacological properties, action pathways and prospectives of humic substances in medicine: an analysis. *Exper*. *Clin*. *Pharm*. **73**(9), 43–48 (in Russian).

Dolgopolov, V.N. 2006. Trials of Gumival™ yield better bovine, swine and fowl stock productivity. In: *Proceedings of conference on summaries and perspectives of humin preparations in livestock industry*. Moscow, p. 40–43 (in Russian).

Dyomin, V.V., Tyeryent'yev, V.A. & Zavgorodnaya, Y.A. 2003. Action pathway of humic substances on living cells. In: *Proceedings of International conference on humus components in the biosphere*. Moscow–Saint Petersburg, pp. 34–35 (in Russian).

EC Joint Research Centre, 1989. ISO/CD 19822 Fertilizers and soil conditioners – Determination of humic and fulvic acids in commercial solid and liquid humic products using alkaline extraction, hydrophobic adsorbent resin and gravimetric determination. *http://www.iso.org/iso/home/store/catalogue_ics/catalogue_detail_ics.htm?ics1=65&ics2=080&csnumber=66271*. Accessed 02.01.2017.

EC Joint Research Centre, 2008. ISO 17321:2008 Fertilizers and soil conditioners – Determination of calcium, sodium, phosphorus, magnesium, potassium, sulphur, iron, zinc, copper, manganese, cobalt and molybdenum by ICP-AES. *http://www.iso.org/iso/home/store/catalogue_ics/catalogue_detail_ics.htm?ics1=65&ics2=080&csnumber=59568*. Accessed 02.01.2017.

EC Joint Research Centre, 2015. ISO 17318:2015 Fertilizers and soil conditioners – Determination of arsenic, cadmium, chromium, lead and mercury contents by AAS. *http://www.iso.org/iso/home/store/catalogue_ics/catalogue_detail_ics.htm?ics1=65&ics2=080&csnumber=59575*. Accessed 02.01.2015.

EUCAST (European Committee on Antimicrobial Susceptibility Testing), 2017. Method for the determination of broth dilution minimum inhibitory concentrations of antifungal agents for conidia forming moulds. *http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/AFST/Files/EUCAST_E_Def_9_3_1_Mould_testing_definitive.pdf*. Accessed 02.01.2017.

Gorbunovskaya, O.M. & Kurzo, V.B. 2001. New methods of sapropel composition analysis. *Chemistry and Chem*. *Eng*. **2**, 73–81 (in Russian).

Gostischeva, M.V., Fyed'ko, I.V. & Posnichenko, Y.S. 2004. A comparative survey of humic acid extraction from peat in humin preparation // *TUSHS Newsletter*: *Autom*. *Systems in Data Proc*. *and Design*, p. 66–69 (in Russian).

ISO/TC 93 (International Organization for Standardization Technical Committee 93), 1997. ISO 10520:1997 Native starch – Determination of starch content – Ewers polarimetric method. *https://www.iso.org/obp/ui/#iso:std:iso:10520:ed-1:v1:en.* Accessed: 03.31.2017.

ISO/TC 93 (International Organization for Standardization Technical Committee 34), 2000. ISO 3596:2000 Animal and vegetable fats and oils – Determination of unsaponifiable matter – Method using diethyl ether extraction. *https://www.iso.org/obp/ui/ #iso:std:iso:3596:ed-1:v1:en.* Accessed: 03.31.2017.

Jacques, K.A. 2003. Ethanol production and the modern livestock feed industry: a relationship continuing to grow. *The Alcohol Textbook*, *4th ed*. 377–385

Kireycheva, I.D. 2006. Chemical nature and biological activity of humic acids. *Study and utilization of peat and sapropel resources*, 315–321 (in Russian).

Kireycheva, L.V. & Khokhlova, O.B. 2000. Elemental composition of humic substances in sapropel deposits. *RAAS Newsletter* **4**, 59–62 (in Russian).

Kosov, V.I. 2007. Sapropel. Resources, Engineering, Geoecology. Saint Petersburg, Nauka, 244 pp. (in Russian).

Kulikova, N.A., Filippova, O.I. Abros'kin, D.P. & Klyain, O.I. 2013. Modern approach to humic substance biological activity assessment. In: *Proceedings of International conference on biodiagnostics in soil ecology analysis*. Moscow, BINOM, pp. 116–119 (in Russian).

Lishtvan, I.I., Kaputskiy, F.N. & Yanuta, Y.G. 2012. Humic acid reactions with metal ions and metal–humic complex structure. *BSU Newsletter* **2**(2), 12–16 (in Russian).

Mushnikova, L.N., Nikiforova, T.A., Sharova, N.Y. & Pozdnyakova, T.A. 2001. Physical, chemical and microbiological composition of carbon mediums for citric acid biosynthesis. *Agricultural Materials Storage and Utilization* **7**, 7–9 (in Russian).

Platonov, V.V., Khadartsev, A.A. Fridzon, K.Y. & Chunosov, S.N. 2014. Chemical composition and biological activity of Glubokoye lake sapropel (Tatarstan). *New Med*. *Eng*. *Newsletter* **3**, 112–118 (in Russian). ISSN 1609–2163.23.

Sharova, N.Y. & Kamen'kova, N.V. 2012. Application of ultrasound and infrared radiation to microbial contamination control in citric acid production // *Agricultural Materials Storage and Utilization*, 4, p. 16–17 (in Russian).

Sharova, N.Y., Kamen'kova, N.V. & Volkova, A.A., 2012. Application of ultrasound and infrared radiation to microbial contamination control in citric acid production. *Agricultural Materials Storage and Utilization* **9**, 31–33 (in Russian).

Shtin, S.M. 2005. Lake Sapropels Complex Utilization. Moscow, MSU, 213 p. (in Russian).

Vybornova, T.V. & Sharova, N.Y. 2015. A citric acid producing strain of Aspergillus niger. Patent RU2558228, C12P 7⁄48, C12N 1⁄14, C12R 1⁄685. (in Russian).

Skurikhin, I.M., prof., MAI member, V.A. Tutel'ian, prof., RAMS member (eds.), 2002. Chemical Composition of Foodstuffs in Russian Market: a Factbook. Moscow, DeLiprint, 236 p. (in Russian).

Vyershinin, V.I. & Pyertsev, N.V. 2005. Planning and Statistical Evaluation of Chemical Experiments: A Reference. Omsk, OmSU, 216 pp. (in Russian).

World Health Organization, 2015. Global Action Plan on Antimicrobial Resistance. *http://www.who.int/iris/bitstream/10665/193736/1/9789241509763_eng.pdf*. Available: 03.31.2017.