

DYNAMICS OF THE MICROBIOLOGICAL INDEXES AT LIME TREATMENT OF SLUDGES FROM A PURIFICATION PLANT

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Abstract. Microbiological indexes have been studied in dynamics at lime treatment and independent planting of lavender (*Lavandula angustifolia*, variety "Hemus") and sweet basil (*Ocimum basilicum*, variety "Genovese") of fresh sludges from a purification plant, with concentrations of quicklime: 10%, 20% and 30% for a 40-day period of composting. The microbiological analysis includes determining of nonpathogenic (non-spore forming bacteria, bacilli, actinomycetes, microfungi, bacteria, which assimilate mineral nitrogen) and pathogenic (*Salmonella* spp., *Escherichia coli* and coliforms, *Enterococcus*, *Clostridium perfringens*) microflora. Out of the beneficial microflora in the sludges the quantity of the non-spore forming bacteria is the biggest, and the lowest is of the actinomycetes. A basic share in the composition of the pathogenic microflora occupies *Clostridium perfringens*, followed by *Escherichia coli* and coliforms, and *Enterococcus*. Absence of *Salmonella* spp. was established in the studied sludges. The best results for the disinfection of the sludges displays the adding of 30% quicklime (followed by the variants with 20% and 30% lime), on 5-th day of the experiment setting. The pH decrease in the period of the study leads to a repeated development of pathogenic microflora. In the meantime the creation of an alkaline medium leads to decrease in the quantity of the beneficial microorganisms. The decrease of the moisture and the drying of the sludges for the study period display a weaker effect on the dynamics of the microflora in comparison with the pH increase impact. The planting of lavender and basil independently in the sludges samples from a purification plant has a beneficial effect (a little bit better with the lavender variants) for increase of the quantity of nonpathogenic microbes and pathogens frequency of occurrence decrease. The effect is intensified by increasing the plant development period.

Keywords: sludges, microflora, lime treatment, lavender, basil

1 Introduction

The sludges from the purification plants are substrata rich in organic substances, which could be used as organic fertilizers in agriculture. The problem for their usage as a fertilizer is the content of heavy metals in them, pathogenic microorganisms and parasites. The lime treatment leads to alkalization of the medium and respectively to sedimentation of the heavy metals in sludges from purification plants, as thus their solubility and mobility are decreased. When calcium oxide is added an exothermic reaction is obtained and the temperature of the sludges is increased, which helps for the destroying of the pathogenic types of microorganisms and parasites, consequently for acceleration of the processes of disinfection and deworming. The hydrated lime forms complexes with smelly sulphurous compounds, like hydrogen sulphide and organic mercaptans (end products from the microbes and parasites activity), as a result of which sediment with smell of calcium ions is obtained. Hence comes the problem with the deodorizing of the sludges, about the solution of which can be used the planting of essential oil crops, some of which also have antimicrobial properties.

According to a study by Marcinkowski (1985) the waste sludges from purification plants, deactivated by quicklime can be used for agricultural purposes. The processing by lime and the pasteurization of the sludges (50-day sanitation) are very efficient methods for disinfection and create safe sludges regarding content of faecal coliforms (Keller et al., 2004). Marinova et al. (2016) present methods for treatment of sludges from purification plants by lime materials. According to these au-

thors the most perspective for a short period of time (around 1 month) is the disinfection of the sludges with adding 20% fine and 30% bigger fraction of quicklime. The treatment by quicklime according to them is especially appropriate for this purpose, since the lime is not dangerous from environmental point of view and in the same time it is effective. In the sludges are isolated pathogenic microorganisms like: *Salmonella* spp., *Listeria* spp., *Escherichia coli*, *Campylobacter* spp., *Clostridium* spp., *Yersinia* spp. and others (Arthurson, 2008; Dudley et al., 1980; Larsen and Munch, 1986; Straub et al., 1993; Strauch, 1991). According to a study by Dermendzhieva (2017) in the sludges from Bulgarian purification plants the number of the faecal (coliforms, *Escherichia coli*) and intestinal pathogenic (*Enterobacteriaceae*, *Salmonella*) microorganisms is over the admissible norms in the Regulations for the order and way of sludges utilization from the purification of waste waters by their usage in agriculture (State Gazette, issue 63/12.08.2016), which makes them unusable (but with potential) for fertilization in the agriculture without applying methods for their disinfection. These microorganisms have strong ability to constantly adapt to the changes in the environment for survival (Kearney et al., 1994) and they can be relatively resistant (especially the spore forming species, like *Clostridium perfringens*) to the frequently used methods for stabilization of sludges (Sahlström, 2003).

The plants synthesize flavonoids as a response of a microbial infection and it is established that these compounds are a mighty antimicrobial agent against a wide spectrum of pathogenic microorganisms (Górniak et al., 2019). In this relation the study of antimicrobial properties of plants at variants with sludges from purification plants, treated for disinfection and deodorizing deserves attention.

The purpose of the study is following the dynamics of microbiological indexes at lime treatment of fresh sludges from a purification plant (in a combination with independent planting of lavender and sweet basil), as a methods of their disinfection of pathogenic microorganisms.

2 Materials and methods

Fresh sludges from a purification plant have been studied as per physico-chemical and microbiological indexes, in dynamics, composted in greenhouse conditions, in plastic packings (containers), treated with quicklime in different concentrations and variants. Lavender (*Lavandula angustifolia*, variety “Hemus”) and sweet basil (*Ocimum basilicum*, variety “Genovese”) are additionally planted independently with three of the variants (table 1):

Table 1. Scheme of the variants and period of microbiological analysis

Variants	Microbiological analysis
Sludge without quicklime (CaO)	Before starting the experiment; Day 1, 6 hours after starting of the experiment; 5th day after starting the experiment; 20th day after starting the experiment; 40th day after starting the experiment
Sludge +10% CaO	
Sludge +20% CaO	
Sludge +30% CaO	
Sludge without quicklime (CaO) + <i>L. angustifolia</i>	
Sludge +10% CaO + <i>L. angustifolia</i>	
Sludge +20% CaO + <i>L. angustifolia</i>	
Sludge +30% CaO + <i>L. angustifolia</i>	
Sludge without quicklime (CaO) + <i>O. basilicum</i>	
Sludge +10% CaO + <i>O. basilicum</i>	
Sludge +20% CaO + <i>O. basilicum</i>	
Sludge +30% CaO + <i>O. basilicum</i>	

Microbiological analyses are carried out as per the method of the limit dilutions and culture on growing media – nonpathogenic microflora: Nutrient agar for non-spore forming bacteria and bacilli; MRS agar for lactobacilli; Actinomycete isolation agar for actinomycetes and bacteria, assimilating mineral nitrogen; Czapek-Dox agar for moulded fungi; pathogenic microflora: Desoxycholate citrate agar for *Salmonella* spp.; Endo agar for *Escherichia coli* and coliforms (oxidase confirming test); ChromoBio Enterococcus agar – for *Enterococcus*; Perfringens agar (TSC and Perfringens selective supplement) for *Clostridium perfringens*. The results are presented like colony-forming units (KOE) a unit substrate. A jar with reagent for generating anaerobic medium is used for the isolation of anaerobes.

For following the temperature of the sludges is used a temperature probe, for determining the moisture – a moisture meter, and for registering pH (in water) – pH meter.

3 Results and discussion

The temperature of the studied sludges is increased to the highest degree on 40-th day from the beginning of the experiment, at adding 30% quicklime (with around 9 °C). By increasing the period of sludges disinfection the moisture decreases by around 1/3 in comparison with the beginning of the experiment (to the highest degree for the variants with 30% lime). The lime treatment leads to alkalization of the medium – to the highest degree on 5-th day after adding 30% lime. On 40-th day from the beginning of the experiment, the sludges values of pH are in the neutral zone again, a little bit higher than the sludges value of pH before the experiment beginning. These trends follow to a weaker degree for the variants with 20% lime and these with 10% lime (table 2).

Table 2. Physico-chemical analyses of raw sludges in dynamics

Variants	Temperature (°C)				
	Before starting	Day 1, 6 hours	5th day	20th day	40th day
Sludge without quicklime (CaO)	20,2	20,4	22,0	24,8	26,4
Sludge +10% CaO		21,1	13,9	26,5	28,1
Sludge +20% CaO		21,6	14,5	26,9	28,7
Sludge +30% CaO		22,2	25,0	27,6	29,4
Sludge without quicklime (CaO) + <i>L. angustifolia</i>		20,6	22,2	24,5	26,1
Sludge +10% CaO + <i>L. angustifolia</i>		20,9	23,4	26,3	27,3
Sludge +20% CaO + <i>L. angustifolia</i>		21,4	23,9	26,7	28,0
Sludge +30% CaO + <i>L. angustifolia</i>		22,0	24,7	27,1	29,1
Sludge without quicklime (CaO) + <i>O. basilicum</i>		20,7	22,4	24,6	26,2
Sludge +10% CaO + <i>O. basilicum</i>		21,0	23,6	26,4	27,7
Sludge +20% CaO + <i>O. basilicum</i>		21,5	24,0	26,8	28,3
Sludge +30% CaO + <i>O. basilicum</i>		22,1	24,9	27,3	29,3
Variants		Humidity (%)			
Variants	Before starting	Day 1, 6 hours	5th day	20th day	40th day
Sludge without quicklime (CaO)	90,0	89,7	84,8	55,7	38,4
Sludge +10% CaO		89,6	84,0	52,0	35,0
Sludge +20% CaO		89,4	85,0	58,0	32,0
Sludge +30% CaO		89,3	70,0	53,0	30,0
Sludge without quicklime (CaO) + <i>L. angustifolia</i>		89,7	83,7	53,8	37,8
Sludge +10% CaO + <i>L. angustifolia</i>		89,3	83,0	50,0	33,0
Sludge +20% CaO + <i>L. angustifolia</i>		89,1	73,0	47,0	31,0

Sludge +30% CaO + <i>L. angustifolia</i>		89,0	68,0	41,0	29,0
Sludge without quicklime (CaO) + <i>O. basilicum</i>		89,8	84,0	56,0	38,0
Sludge +10% CaO + <i>O. basilicum</i>		89,4	83,4	51,0	34,0
Sludge +20% CaO + <i>O. basilicum</i>		89,2	73,5	47,4	32,0
Sludge +30% CaO + <i>O. basilicum</i>		89,1	68,5	41,6	29,6
	pH (in water)				
Variants	Before starting	Day 1, 6 hours	5th day	20th day	40th day
Sludge without quicklime (CaO)	6,88	6,88	6,90	6,89	6,90
Sludge +10% CaO		10,88	11,23	9,12	7,18
Sludge +20% CaO		11,21	12,05	9,31	7,31
Sludge +30% CaO		11,78	12,37	9,43	7,43
Sludge without quicklime (CaO) + <i>L. angustifolia</i>		6,88	6,92	6,93	6,93
Sludge +10% CaO + <i>L. angustifolia</i>		10,91	11,26	9,12	7,20
Sludge +20% CaO + <i>L. angustifolia</i>		11,25	12,15	9,36	7,33
Sludge +30% CaO + <i>L. angustifolia</i>		11,82	12,42	9,47	7,44
Sludge without quicklime (CaO) + <i>O. basilicum</i>		6,88	6,91	6,90	6,91
Sludge +10% CaO + <i>O. basilicum</i>		10,89	11,25	9,13	7,18
Sludge +20% CaO + <i>O. basilicum</i>		11,23	12,11	9,33	7,32
Sludge +30% CaO + <i>O. basilicum</i>		11,80	12,40	9,44	7,43

The microbiological analysis of the sludges from the purification plant has shown presence of specific and non-specific (pathogenic) microflora. The quantity of the nonpathogenic groups microorganisms is presented in the next table 3.

Table 3. Quantity and quality content of the nonpathogenic microflora in sludges in dynamics

Variants	Non-spore-forming bacteria (c.f.u/g)					
	Before starting	Day 1, 6 hours	5th day	20th day	40th day	
Sludge without quicklime (CaO)	2000	2000	2480	2300	2000	
Sludge +10% CaO		1900	1100	1440	1600	
Sludge +20% CaO		1820	900	1220	1480	
Sludge +30% CaO		1740	720	1080	1200	
Sludge without quicklime (CaO) + <i>L. angustifolia</i>		2000	2520	2400	2300	
Sludge +10% CaO + <i>L. angustifolia</i>		1900	1100	1500	1720	
Sludge +20% CaO + <i>L. angustifolia</i>		1820	900	1340	1580	
Sludge +30% CaO + <i>L. angustifolia</i>		1740	720	1220	1320	
Sludge without quicklime (CaO) + <i>O. basilicum</i>		2000	2500	2360	2240	
Sludge +10% CaO + <i>O. basilicum</i>		1900	1100	1480	1700	
Sludge +20% CaO + <i>O. basilicum</i>		1820	900	1300	1520	
Sludge +30% CaO + <i>O. basilicum</i>		1740	720	1180	1280	
		Bacilli / Lactobacilli (c.f.u/g)				
Variants		Before starting	Day 1, 6 hours	5th day	20th day	40th day
Sludge without quicklime (CaO)		520	600	580	560	

Sludge +10% CaO	520	500	440	500	540
Sludge +20% CaO		460	400	460	500
Sludge +30% CaO		420	360	420	480
Sludge without quicklime (CaO) + <i>L. angustifolia</i>		520	640	620	600
Sludge +10% CaO + <i>L. angustifolia</i>		500	440	540	580
Sludge +20% CaO + <i>L. angustifolia</i>		460	400	520	540
Sludge +30% CaO + <i>L. angustifolia</i>		420	360	460	500
Sludge without quicklime (CaO) + <i>O. basilicum</i>		520	620	600	580
Sludge +10% CaO + <i>O. basilicum</i>		500	440	520	560
Sludge +20% CaO + <i>O. basilicum</i>		460	400	480	520
Sludge +30% CaO + <i>O. basilicum</i>		420	360	440	480
Actinomycetes (c.f.u/g)					
Variants	Before starting	Day 1, 6 hours	5th day	20th day	40th day
Sludge without quicklime (CaO)	80	80	100	80	80
Sludge +10% CaO		40	0	60	80
Sludge +20% CaO		20	0	40	60
Sludge +30% CaO		0	0	20	40
Sludge without quicklime (CaO) + <i>L. angustifolia</i>		80	120	120	140
Sludge +10% CaO + <i>L. angustifolia</i>		40	20	100	120
Sludge +20% CaO + <i>L. angustifolia</i>		20	0	80	80
Sludge +30% CaO + <i>L. angustifolia</i>		0	0	60	60
Sludge without quicklime (CaO) + <i>O. basilicum</i>		80	100	100	120
Sludge +10% CaO + <i>O. basilicum</i>		40	20	80	100
Sludge +20% CaO + <i>O. basilicum</i>		20	0	60	60
Sludge +30% CaO + <i>O. basilicum</i>		0	0	40	60
Micromycetes (c.f.u/g)					
Variants	Before starting	Day 1, 6 hours	5th day	20th day	40th day
Sludge without quicklime (CaO)	640	640	620	600	580
Sludge +10% CaO		540	520	540	560
Sludge +20% CaO		500	460	480	500
Sludge +30% CaO		460	420	460	480
Sludge without quicklime (CaO) + <i>L. angustifolia</i>		660	620	640	660
Sludge +10% CaO + <i>L. angustifolia</i>		540	540	580	600
Sludge +20% CaO + <i>L. angustifolia</i>		500	500	520	540
Sludge +30% CaO + <i>L. angustifolia</i>		460	460	500	520
Sludge without quicklime (CaO) + <i>O. basilicum</i>		660	620	620	640
Sludge +10% CaO + <i>O. basilicum</i>		540	540	560	580
Sludge +20% CaO + <i>O. basilicum</i>		500	480	500	520
Sludge +30% CaO + <i>O. basilicum</i>		460	440	480	500
Bacteria, digesting mineral nitrogen (c.f.u/g)					
Variants	Before starting	Day 1, 6 hours	5th day	20th day	40th day
Sludge without quicklime (CaO)		1920	2280	2100	2020

Sludge +10% CaO	1920	1900	1200	1380	1540
Sludge +20% CaO		1860	1000	1240	1500
Sludge +30% CaO		1780	900	1040	1360
Sludge without quicklime (CaO) + <i>L. angustifolia</i>		1920	2340	2140	2080
Sludge +10% CaO + <i>L. angustifolia</i>		1900	1260	1420	1580
Sludge +20% CaO + <i>L. angustifolia</i>		1860	1040	1280	1560
Sludge +30% CaO + <i>L. angustifolia</i>		1780	940	1080	1400
Sludge without quicklime (CaO) + <i>O. basilicum</i>		1920	2300	2120	2040
Sludge +10% CaO + <i>O. basilicum</i>		1900	1220	1400	1560
Sludge +20% CaO + <i>O. basilicum</i>		1860	1020	1260	1540
Sludge +30% CaO + <i>O. basilicum</i>		1780	920	1060	1380

The quantity of the non-spore forming bacteria and bacteria, assimilating mineral nitrogen is the highest, and the actinomycetes are presented the least (to complete absence with lime treatment). The lime treatment increases the pH values of the sludges (alkaline medium, to pH around 12), which leads to decrease of the quantity of the analyzed nonpathogenic groups microorganisms. On the other hand the decrease in the moisture of the sludges increases the quantity of the microorganisms. The moisture and the temperature of the substrata are of essential importance for the development of the microbes. The excessively high amount of moisture in the sludges (90%) in the beginning of the experiment limits in a certain degree the development of the microorganisms. The bacilli as spore-forming species and micromycetes as requiring higher moisture survive well in extreme conditions – as per amount in the content of the microflora they are after the non-spore forming bacteria and the bacteria, which assimilate mineral nitrogen.

The lime treatment renders stronger limiting impact over the development of the nonpathogenic groups of microorganisms in comparison with the decrease of the moisture (from 90% up to 30-38%) and the drying of the sludges by increase in the temperature with around 6 °C up to 9 °C in the end the experiment (the amount of the microbes with the variants without lime treatment is higher than the one with the variants with lime treatment). The results show that the adding of 30% quicklime decreases the amount of the microorganisms to the highest degree – the effect follows for the variants with 20% lime and least impact over the amount of the microbes renders the adding of 10% lime in the sludges. This trend regarding the dynamics in the amount of the microorganisms for the period of study, is preserved for the variants up to 5-th day from the setting of the experiment, until the sludges are with stronger alkaline medium (pH 11-12). The decrease of pH to a weaker alkaline medium (pH around 9,1-9,4) on 20-th day and a neutral medium (pH 7,2-7,4) on 40-th day increases the amount of all analyzed nonpathogenic groups of microorganisms. As a whole the amount of the microorganisms is a little bit higher with the variants with lavender in comparison with the variants with basil, followed by the ones without vegetation, regardless of the added amount of lime and the decrease of moisture in the period of analysis.

The lime treatment is a method which is used for disinfection (destroying of pathogenic microorganisms) of the sludges from purification plants. In the studied variants were established: *Escherichia coli* and coliforms, Enterococcus, *Clostridium perfringens* (figure 1 up to figure 3).

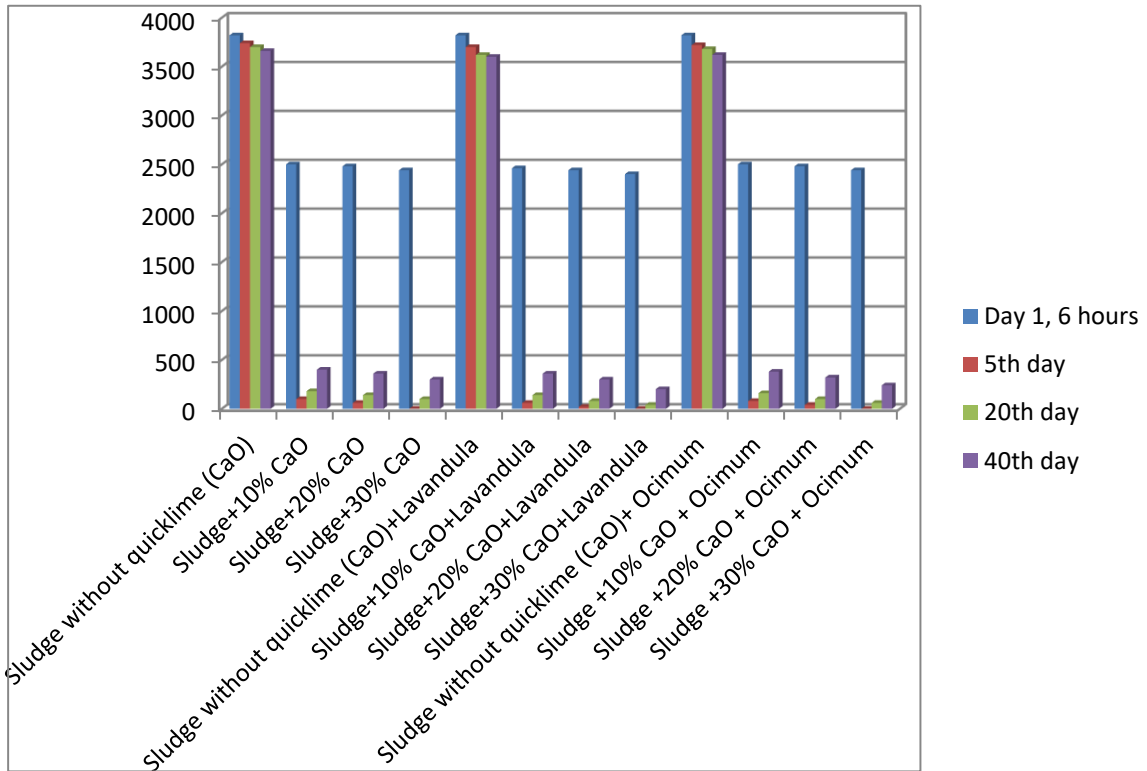


Fig. 1. *Escherichia coli* and coliforms (c.f.u/g)

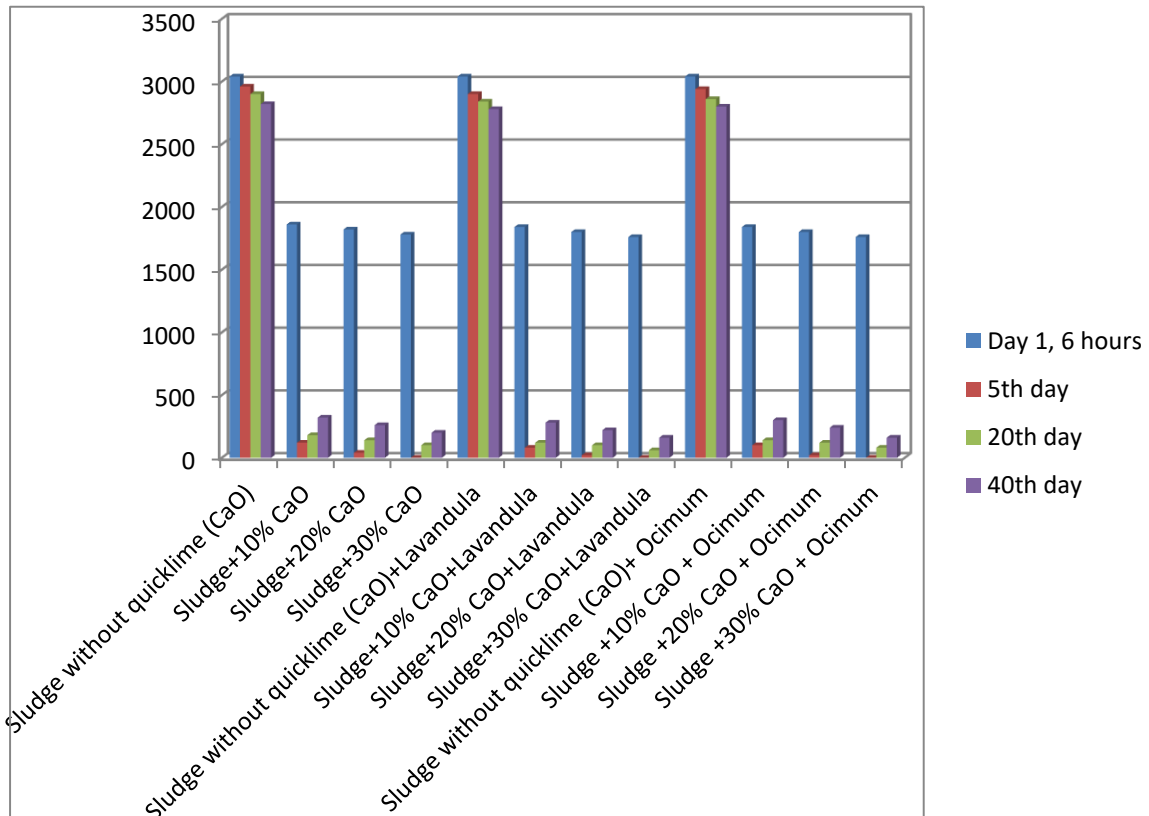


Fig. 2. *Enterococcus* (c.f.u/g)

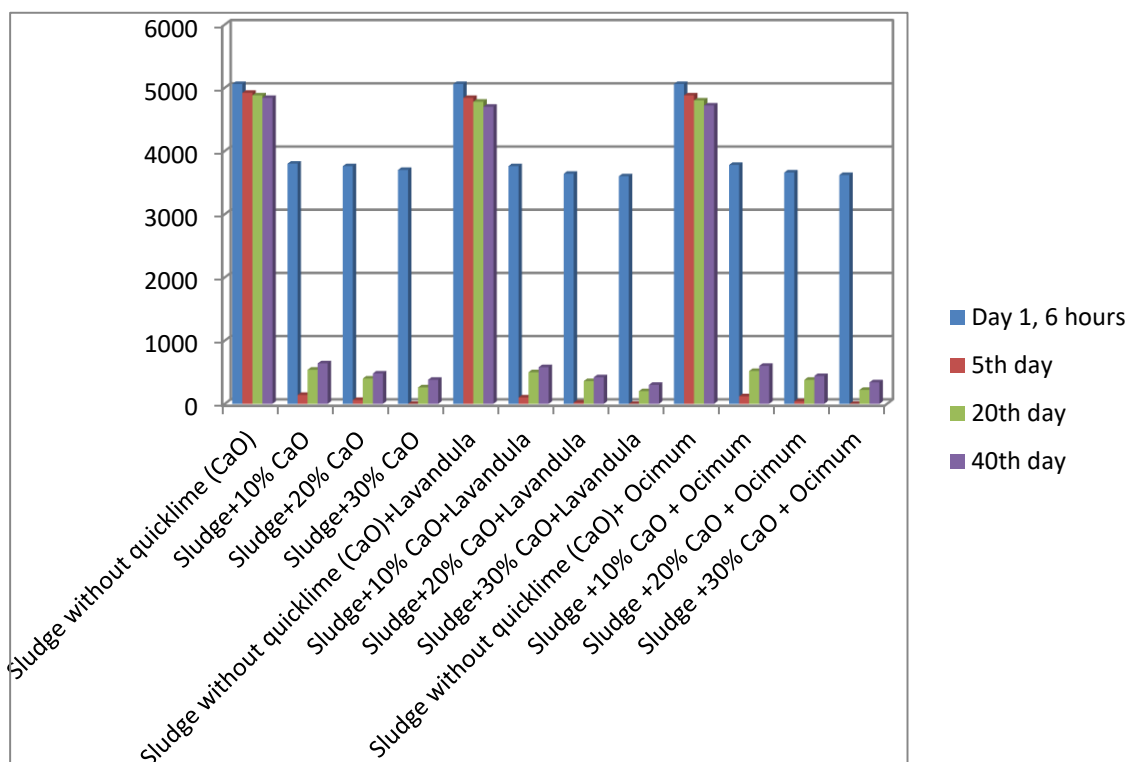


Fig. 3. *Clostridium perfringens* (c.f.u/g)

In the content of the pathogenic species in the sediment the frequency of occurrence of *Clostridium perfringens* (5060 cfu/g before beginning of the experiment) is the highest, followed by *Escherichia coli* and coliforms (3820 cfu/g before beginning of the experiment), and *Enterococcus* (3040 cfu/g before beginning of the experiment). Absence of *Salmonella* spp. is established. *Clostridium perfringens* is a spore forming species, which impede its destroying. The lime treatment leads to decrease in the quantity of the pathogenic microbes (at all variants with lime treatment) and their complete destroying until 5-th day from setting the experiment at the variants with 30% lime, regardless of the vegetation. Marinova et al. (2016) also establish best disinfection of sludges from purification plants at adding 20% and 30% quicklime. According to the study of these authors for 31 days after the mixing of the sludges with fine quicklime in the indicated concentrations, *Clostridium perfringens* is destroyed. The results of their studies confirm that for obtaining safe sediment from an epidemiological point of view, it should be processed for one month or a shorter period. The same authors have established that the massiveness of the quicklime also renders a different impact over the development of the microorganisms – the larger fraction homogenises more difficult with the sediment, because of which the usage of a higher norm is necessary. Therefore the creation of an alkaline medium is a method for the sediment purification of pathogenic microorganisms. The pH values decrease, however, (a weaker alkaline medium – 20-th day and a neutral medium – 40-th day) leads to a repeated development of pathogenic microorganisms – to a higher degree for *Clostridium perfringens*. The adding of 30% lime and the planting of lavender display best results for the destroying of the pathogenic microorganisms. The variants with 30% lime and basil follow as per efficiency, after them the variants without vegetation. The decrease of the moisture and the drying of the sludges have significantly weaker effect than the lime treatment, which is indicative of the fact that at the variants without lime treatment the results are preserved close for the whole period of the study. The conditions of the medium, as the active reaction of the sludges, the salt content, the heavy metals content, temperature, can lead to a change in the progress of the microbial activity in the substrate and respectively to influence on the reproductive ability of the microorganisms (Dermendzhieva, 2017). The studied pathogenic microorganisms have strong ability to adapt constantly to the changes in the environment in order to survive (Kearney et al., 1994) and they can be relatively resistant (especially the spore forming species, like *Clostridium perfringens*) to the frequently used methods for stabilization of sludges (Sahl-

ström, 2003). A positive effect for disinfection of sludges from purification plants by lime treatment applying is established by other scientists either (Marcinkowski, 1985; Keller et al., 2004). On the one hand, for the complete destroying and prevention of repeated development of the pathogenic microflora is necessary an additional lime treatment after 5-th day for preservation of the alkaline medium, but on the other hand this would lead to decrease in the quantity of the useful microorganisms. Therefore, a combination of lime treatment with ash adding, microbial fertilizers, appropriate plant species and other methods are necessary in a process of repeated analyses and following the development of the microflora in the sludges in dynamics.

4 Conclusions

Fresh sludges from a purification plant have been studied after lime treatment and independent planting of lavender and basil for a period of 40 days regarding dynamics of nonpathogenic and pathogenic microorganisms. Non-spore forming bacteria, bacilli, including lactobacilli, actinomycetes, micromycetes, bacteria, assimilating mineral nitrogen, *Salmonella* spp., *Escherichia coli* and coliforms, *Enterococcus*, *Clostridium perfringens* have been analysed. Out of the beneficial microflora in the sludges the highest is the quantity of the non-spore forming bacteria, and the lowest of the actinomycetes. In the content of the pathogenic microflora a basic share occupy *Clostridium perfringens*, followed by *Escherichia coli* and coliforms, and *Enterococcus*. The absence of *Salmonella* spp. is established in the studied sludges.

The lime treatment renders a beneficial effect for the destroying of pathogenic microorganisms in samples with sludges from a purification plant. Best results for their disinfection has the adding of 30% quicklime (follow the variants with 20% and 10% lime), on 5-th day after setting of the experiment. In the same time, however, the creation of an alkaline medium leads to decrease in the quantity of the useful microorganisms. On the one hand, an additional lime treatment after 5-th day is necessary for a complete destroying and prevention of the pathogenic microbes' repeated development (especially for the spore forming species *Clostridium perfringens*), and on the other hand, probably this would lead to decrease of the beneficial microorganisms to a higher degree.

The temperature of the studied sludges is increased by around 6 °C up to 9 °C, and the moisture decreases by 1/3 until the 40-th day from beginning of the experiment, to the highest degree at adding 30% quicklime. The lime treatment leads to alkalization of the medium – to the highest degree on 5-th day after adding 30% lime. On 40-th day from the beginning of the experiment, the pH values of the sludges decrease to a neutral medium. These trends follow to a weaker degree for the variants with 20% lime and these with 10% lime. The decrease of the moisture and drying of the sludges for the period of study displays a weaker effect on the dynamics of the pathogenic, as well as of the nonpathogenic microorganisms in comparison with the impact of the pH increase.

The independent planting of lavender and basil in the samples of sludges from a purification plant has a favourable effect (a little bit better with the variants with lavender) for increase in the nonpathogenic microbes quantity and decrease of the frequency of occurrence of the pathogenic ones. The effect increases by increase in the period for development of the plants. The selection of these plant species is appropriate for disinfection and deodorizing of the sludges in a combination with lime treatment.

Continued studies and a multifactor approach are necessary for establishment of appropriate combined variants simultaneously for complete disinfection of the sludges and preservation of the beneficial microflora, with purpose their safe usage as an organic fertilizer in agriculture.

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