

DETECTING MICROORGANISMS IN SEWAGE VIA METHOD OF GAS CHROMATOGRAPHY (GC) WITH MASS SPECTROMETRY (MS)

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The article presents the results of studying sewage before mechanic cleaning and silt from silting grounds of Govsaninskaya station of Baku with bacterial flora. A spectre of aerobic and anaerobic microorganisms has been received. It has been established via express method of detection – chromatography with mass spectrometry. Expression, sensitivity, and informativity of the method, and it can be utilized for monitoring infections from various positions.

Keywords: sewage, silt, detection, bacterial flora, chromatography with mass spectrometry

The main purpose of sanitary-microbiological research of sewage is a supervision over an efficiency of cleaning and disinfection of sewage and their sediments before introducing them into pools, seas, and irrigation fields.

According to the existing regulative documents, in a number of cases uncleaned water or water at the stage of cleaning before chloration is studied for presence of lactose-positive bacterias, and after chloration bacterias of Enterobacteriaceae family are defined for a control of operation of cleaning facilities [1, 2].

But, considering the fact that in terms of Azerbaijan sewage are introduced into the sea after cleaning and it is also used in irrigation, and silt deposits are used as fertilizers, there is a necessity for a new, accelerated and broadened control over water cleaning.

Our claims are based on previous researches on studying viral and bacterial content of sewage that have revealed a wide spectre of viral and bacterial population of microorganisms [3, 4, 5, 6].

The research has been carried out via classic general bacteriological and virological methods of indication and identification that, as known, are informative, but labourious, expensive, and long.

Considering the mentioned information, target objective of this research is to reveal a spectre of bacterial flora in sewage and active silt at silting grounds of Govsaninskaya cleaning station of Baku, with processing and utilizing gas chromatography with mass spectrometry for detecting microorganisms.

Materials and methods of research

Probes of sewage, selected before mechanic cleaning and probes of active silt from silting grounds of Govsaninskaya cleaning station were selected as materials of the research.

Detection of bacterial microorganisms was carried out via method of gas chromatography with mass spectrometry [7, 8, 9].

Method of gas chromatography is one of the modern analytic instrumental methods.

Data bank of chemical components of clinically-significant microorganisms (NIST) served as a basis for defining kinds of microorganisms.

Using chemical composition of microorganism cells to identify kinds of microorganisms for their medical diagnostics lies in a basis of this method.

An analysis is preceded by processing probes of sewage – its centrifugation under 2500 rpm during 30 minutes in order to sediment large disperse fractions. Then comes a period of probe preparation – discharge of fractions of lipid acids and their transition into compositions, suitable for chromatographic analysis.

After biopreparation, 0,4 ml of probe is dried with addition of small amounts of methanol (0,2 ml).

Then, 0,2 ml of 1,7 M HCl is added into metanol and methanolysis under 80 degrees of celcium is carried out during an hour.

As a result of reaction of methanolysis, LA (lipid acids) are freed as methylic ethers of lipid acids. These components are extracted two times with hexane – 200 mcl (microliters).

Reaction solution in quantity of 2 mcl is introduced into an injector of chromate mass spectrometer GCMS-QP-2010S (Shimadzu, Japan).

Chromatographic division has been carried out on a capillary column 50 m long, 0,32 mm in diameter, with film thickness of 0,53 micron.

Chromatography regime: 120 degrees, 2 minutes, 15 degrees/minute up to 230 degrees. Mass spectrometer – quadrupole, with ionization and electric impact (70 eq).

Interpretation of data of mass fragmento grammes consists in defining peaks' belonging to corresponding substances according to their mass and time of chromatographic hold (Ret. Time). It is defined according to chromatogram me in scanning regime, when it is possible to define a nature of chemical component of a cell.

Comparison was carried out according to NIST library.

Results of research and their discussion

The result of chromatographic analysis of sewage probes has established a presence of markers of bacterial flora of wide spectre (Fig. 1) that corresponds to a certain type of microorganisms (table 1).

As you can see in Fig. 1, each component of a solution corresponds to a separate peak on chromatogramme. Time period from the begin-

Defining quantitative composition of a solution is based on an assumption that an intensity of a peak of each component is proportional to its content in the solution.

Peak area S (Area $\mu S'$ min) is used as a measure of intensity.

Normally, a height of a peak h is multiplied by its width W , measured at the middle of a peak's height: $S = hw$.

Utilizing mass spectrometer as a detector of gas chromatograph allows us not only to register presence of a divided component in it, but also establish its structure.

A wide usage of chromat mass spectrometers has actualized a necessity to create giant libraries, databases of mass spectres of various solutions.

Speaking of advantages of mass spectrometry we first of all should outline a sensitivity,

expressivity, informativity, and reliability of the method.

This method is a universal method of indication that is equally effective for aerobic and anaerobic microorganisms (G.A. Osipov, 1996) [9].

Thus, the following microorganisms' markers have been revealed in sewage: general coliforms, *Streptococcus faecalis*, *Pseudomonas aeruginosae*, *Bacillus subtilis*, *Clostridium perfringens*, *Peptostreptococcus*, *Bifidobacterium*, *Staphylococcus*, *Actinomyces*, *Propionibacterium*. A similar spectre of markers has been revealed while studying active silt that is used for inactivating microorganisms in sewage according to phenomenon of interference of saprophytic factor μ (table 2, Fig. 2)

Table 2

Spectre of bacterial microorganisms that have been detected in active silt of Govsaninsjaya cleaning station according to markers of NIST

Peak name	Ret. time min	Area $\mu S'$ min	Ret area%	Types of microorganisms
2 Pentene, 2,3 dimethyl	6,825	4425	0,881	All microorganisms
Pentanoic acid, 4- oxo methyl, ester	10,073	104865	25,556	General coliforms
2,4 Hexadienedioic acid demethyl ester $C_8H_{12}O_2$	14,003	41807	9,316	E. coli
Hexadienedioic acid methyl ester	15,096	63265	12,597	General coliforms
Octadecanoic acid methyl- ester $C_{19}H_{38}O_2$	23,452	58532	16,261	All microorganisms
11 octadecanoic acid methyl- ester $C_{18}H_{38}O_2$	24,385	51995	14,445	<i>Bacillus subtilis</i>
10 octadecanoic acid methyl- ester $C_{18}H_{38}O_2$	24,636	2246	0,624	<i>Clostridium perfringens</i>
13 octadecanoic acid methyl- ester	24,822	5095	0,514	<i>Peptastreptococcus faecalis</i>
9.12 Octadecanoic acid methyl- ester	26,290	2564	0,986	<i>Peptostreptococcus</i> , <i>Bifidobacterium</i>
Cis 11 eicosenoic acid methyl ester	33,741	2307	0,432	<i>Staphylococcus aureus</i> , <i>Actinomyces</i> <i>Propionibacterium</i>

To conclude, we should say that results of our research in field of hygiene and microbiology of environment give us ground to claim that utilizing methods of chromatography with mass

spectrometry as an express method of detecting microorganisms in an environment is purposeful for control over cleaning sewage at cleaning stations and in specific epidemic situations.

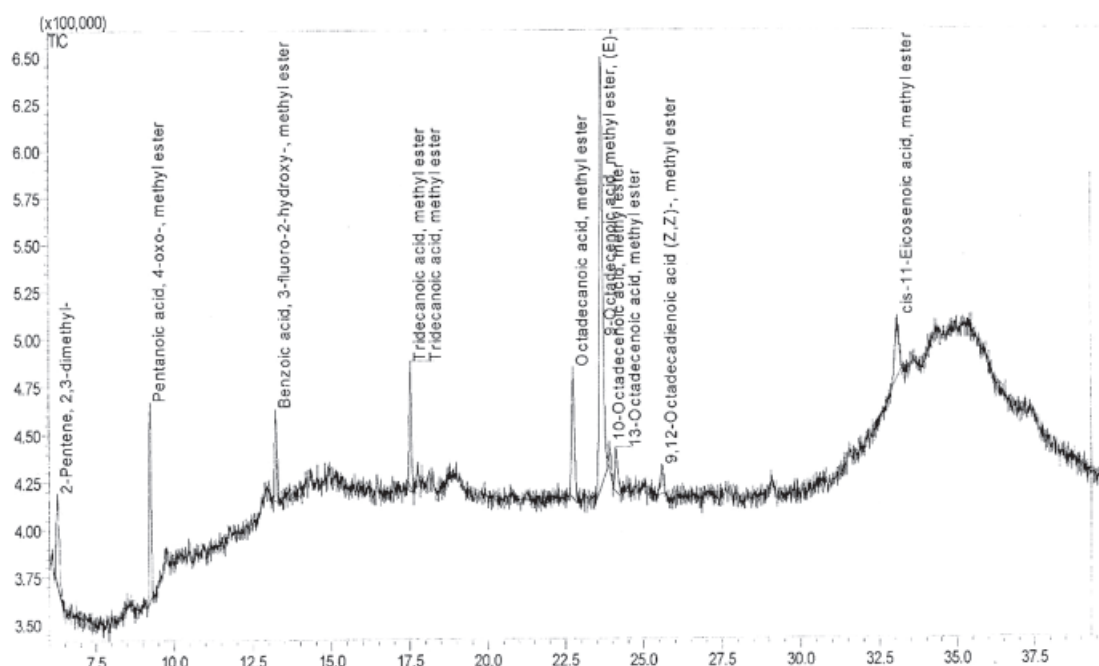


Fig. 2. Chromatogramme of active silt probes

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