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Abstract: The main problem in the development of acousto-electronic gas sensors is the search of materials which are sensitive to the presence of different gases. For this purpose in this paper we suggest the use of piezoelectric quartz resonators coated by the mycelium films of mushroom Lentinula edodes strain F-249 which were cultivated in a synthetic medium; the extract of mycelium was deposited on the surface of the resonator. Measurement of the frequency dependence of the complex electric impedance of the resonator allowed us to evaluate the density, elastic constants and viscosity of the films under test. The influence of different gases, such as ammonia, formaldehyde, ethylacetate and volatile liquids: acetone, acetic acid, chloroform on the physical properties of the extracts of mushroom Lentinula edodes mycelium has been investigated. Results have shown that this material, prepared according to different technological procedures, is suitable as a sensitive layer for the detection of ammonia, formaldehyde and ethylacetate.

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Dear Sir/Madam,

We would like to submit for publication in Sensors and Actuators B our manuscript entitled "Acoustoelectronic Gas Sensor Based on Mushroom Mycelial Extracts" authored by Iren E. Kuznetsova, Boris D. Zaitsev, Alexander M. Shikhabudinov, Olga M. Tsivileva, Alexei N. Pankratov, EnricoVerona.

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Thank you for your consideration of this paper.

Sincerely,

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Acousto-electronic Gas Sensor Based on Mushroom Mycelial Extracts

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Abstract. The main problem in the development of acousto-electronic gas sensors is the search of materials which are sensitive to the presence of different gases. For this purpose in this paper we suggest the use of piezoelectric quartz resonators coated by the mycelium films of mushroom *Lentinula edodes* strain F-249 which were cultivated in a synthetic medium; the extract of mycelium was deposited on the surface of the resonator. Measurement of the frequency dependence of the complex electric impedance of the resonator allowed us to evaluate the density, elastic constants and viscosity of the films under test. The influence of different gases, such as ammonia, formaldehyde, ethylacetate and volatile liquids: acetone, acetic acid, chloroform on the physical properties of the extracts of mushroom *Lentinula edodes* mycelium has been investigated. Results have shown that this material, prepared according to different technological procedures, is suitable as a sensitive layer for the detection of ammonia, formaldehyde and ethylacetate.

Keywords: mycelium films, *Lentinula edodes*, quartz resonator, electric impedance, equivalent circuit, acoustic gas sensor

1. Introduction

Currently, due to the increasing role of ecological and biological safety and the need to prevent or minimize the consequences of terrorist attacks and industrial accidents the further development and improvement of existing chemical sensors remain urgent. These sensors may be based on various different physical principles, including acousto-electronics. During the last decade a great number of different methods and approaches for the development of acoustical chemical sensors for monitoring gaseous and liquid environments have been proposed [1, 2]. Presently there exists a large amount of papers suggesting the use, as chemical sensors, of piezoelectric resonators [3] and surface [4] or plate [5] acoustic wave delay lines. Most of the acoustic chemical sensors are based on use of specific films applied to the surface of the acoustic propagation medium, whose physical properties are affected by exposure to specific chemical analites. These, in turn, affect the characteristics of the acoustic wave specifically phase velocity and/or damping, whose changes give information about the presence of a chemical agent in the environment. Both organic and inorganic materials have been exploited as sensitive films, including ZnO, TeO_2, SnO_2, and TiO_2 [6, 7], polymeric materials [8, 9], films of polypyrrole [10], carbon nanotubes [11], graphene –like materials [12, 13] and various nanoparticles [14]. Nevertheless the problem of searching for gas sensitive materials showing high performances as to sensitivity and selectivity to given gases, together with reversible and repeatable operation is still a target of prime importance.

One of the opportunities yet poorly studied is the use of extracts from higher fungi. Earlier it was experimentally stated that several mushrooms extracts exhibited high sorption sensitivity to the phenol and water vapor. As a result of this study, on the basis of extracts from *Pleurotus ostreatus* (oyster mushroom), the modifier for electrodes of the resonator-type piezo-sensor was developed, which was characterized by a high sensitivity to phenol vapor in gaseous phase, rapid response, and applicable determination error [15]. However, a research on the sorption sensitivity of extracts obtained from mushrooms at different developmental steps was not performed as yet; furthermore, the sorption sensitivity of mushroom mycelia to other volatile liquids and gases was not explored so far. Unexplored in this respect are the mycelial extracts of other higher fungi. The present work deals with studying the feasibility of implementing films obtained by solvent volatilization from the mushroom mycelial extracts, as a sensitive coating for acoustoelectronic sensors.

In order to evaluate the possibility of using the mycelium films of higher fungi as a gas sensitive membrane, at first the methods of their preparation and the evaluation of their mechanical properties were at first carried out. Then techniques for analyzing the changes produced on these properties by the interaction with the gas where developed, as well as the most suitable conditions for the production of films showing a fast and reversible behavior after exposure to the chemical agent.

2. Experimental

2.1. Preparation of thin mycelial mats

To study the characteristics of the film experimentally, samples of the basidiomycete *Lentinula edodes* (shiitake mushroom) mycelium were first got. We used a culture of *Lentinula edodes* (strain F-249) obtained from the collection of higher basidiomycetes of the Department of

Mycology and Algology, Moscow State University. The fungal culture was maintained on wort agar at 4°C. As an inoculum, a 14-day culture of *Lentinula edodes* grown at 26°C on beer-wort agar (4° Brix) was used. From the resulting mycelium, using a metal punch with a diameter of 5 mm under sterile conditions, we cut-out blocks, and inoculated liquid nutrient media at a rate of two blocks for 20 ml of medium. The submerged mycelium of the mushroom was grown in a liquid synthetic glucose-asparagine medium (9 g/l of *D*-glucose and 1.5 g/l of *L*-asparagine). The compounds of indolic nature (indolyl-3-acetic acid (IAA) or tryptophan) were added as solutions in ethanol-H_2O (1:1, *v/v*) mixtures into the autoclaved nutrient media immediately before seeding under sterile conditions. The concentrations of indolic compounds in the culture medium were 0.2, 0.5, 1.0, 5.0, 10, and 100 mg/l. Our work [16] related to the effects of IAA and tryptophan, along with other indolics, on the submerged mushroom culture argued in favor of choosing these indolic additives. The extraction of shiitake mycelium was performed according to three different procedures, using: 1) distilled water; 2) 96% (*v/v*) aqueous ethanol; 3) 50% (*v/v*) aqueous ethanol. As a result, the samples obtained were a kind of suspension consisting of the mycelial biomass and the extracting material.

2.2. The composite resonator loaded by the film under study

Experiments were carried out exploiting a standard thickness shear resonator (AT –cut quartz) with longitudinal electric field and a resonant frequency of ~3 MHz. The diameter of the electrodes was 6 mm and the thickness of the quartz plate 0.5 mm. The resonator was mounted on a commercial holder ensuring, at the same time, reliable electric contacts together with the required mechanical strength (see Fig.1 a and b). The solution of the mycelium extract with a volume of 4 µl with the help of a measuring pipette was deposited on one of the resonator electrodes. Such amount of solution allowed to cover the electrode completely without any spread out of the metallized area. The resonator was then kept in air for 24 h so to dry the solution and obtain the mycelium film. In this way thin, ≈ 10 µm thick, transparent films of mycelium extract were obtained.

2.3. Test chamber

The sorption sensitivity tests of mycelium films, under laboratory conditions, were performed into a special chamber, specifically designed to provide calibrated gas mixtures as shown in Fig. 1,c. The chamber, including the retort for volatile liquid, consisted of a glass cylinder hermetically closed by a glass cover with a teflon sealing. The resonator under test was set inside the chamber with the contact rods hermetically sealed trough the chamber walls. Experiments were carried out by filling the retort with the volatile liquid to test and closing the cylinder by the glass cover; in a few minutes the liquid was completely evaporated, filling up the chamber. A special attention was paid to the electrical quality of the contacts between the resonator and the measuring set up.

2.4. Measurement of physical characteristics of the films

At first the frequency dependency of the real (R) and imaginary (X) parts of the complex electric impedance for the bare quartz resonator was measured by means of a precision LCR meter (HP 4285A, Agilent Technologies, Santa Clara, CA). The Mason's equivalent circuit of the unloaded resonator, that has been exploited to calculate the theoretical frequency dependence of the impedance is shown in Fig. 2a [17, 18]. The circuit includes the contribution of both the quartz plate and electrodes, whose thickness is comparable to that of the films to be analyzed. The mechanical impedances Z_1, Z_2 , Z_{1m} and Z_{2m} in the equivalent circuit of Fig.2 may be expressed as:

$$\begin{aligned} Z_1 &= i \cdot Z_p S \tan\left(\frac{k_p d_p}{2}\right); \qquad Z_2 = -i\frac{Z_p S_p}{\sin\left(\frac{kd}{2}\right)}; \\ Z_{1m} &= i \cdot Z_m S \tan\left(\frac{k_m d_m}{2}\right); \quad Z_{2m} = -i\frac{Z_m S}{\sin\left(\frac{k_m d_m}{2}\right)}; \end{aligned} \tag{1}$$

with $Z_p = \left(c_{66}^{(p)}\rho_p\right)^{1/2}$ and $Z_m = \left(c_{66}^{(m)}\rho_m\right)^{1/2}$ specific acoustic impedances of the piezoelectric medium and electrodes of thickness d_p, and d_m, respectively. $c_{66}^{(p)}$, $c_{66}^{(m)}$, ρ_p, and ρ_m are the shear elastic constant and density in the two media, while $k_p = \omega/v_p$ and $k_m = \omega/v_m$ are the respective wave numbers, being ω the angular frequency. v_p and v_m are the acoustic phase velocities in the quartz plate and electrodes, and finally S is the area of the electrodes while i is the imaginary unit. The electro-mechanical transformer in the equivalent circuit takes into account the mutual transformation of electrical and mechanical energy [17, 19]; its transformation ratio N is given by $N = hC_0$, where C_0 represent the static capacitance of the piezoelectric plate coated with the metal electrodes: $C_0 = \varepsilon_p S/d_p$ with ε_p absolute electric permittivity of the piezoelectric plate, and $h = e_p/\varepsilon_p$ where e_p is the piezoelectric constant responsible for the electromechanical coupling.. Viscosity of the piezoelectric material η_{66} was considered as the main source of losses. Under this assumption the velocities and the specific acoustic impedances in the resonator and electrodes may be written as [19]:

$$v_p = \sqrt{\frac{c_{66}^{(p)} + \frac{e_{25}^2}{\varepsilon_{11}} + i\omega\eta_{66}}{\rho_p}}, \quad Z_p = \sqrt{\left(c_{66}^{(p)} + \frac{e_{25}^2}{\varepsilon_{11}} + i\omega\eta_{66}\right)\rho_p} \tag{2}$$

$$v_m = \sqrt{\frac{c_{66}^{(m)}}{\rho_m}}, \quad Z_m = \sqrt{c_{66}^{(m)}\rho_m} \tag{3}$$

Kirchhoff's equations have been applied to the equivalent circuit of Fig. 2a to calculate the frequency dependency of the real and imaginary parts of the resonator impedance for the specific material constants considered [18]. These constants where allowed to vary within a limited range and the least-squares method exploited to find a set of material constants providing the closer agreement between the theoretical and experimental impedance.

After mycelium film coating and drying, the equivalent circuit describing the resonating structure is that of Fig. 2b. The acoustic impedances Z_{1f} and Z_{2f} of the mycelium film are:

$$Z_{1f} = i \cdot Z_f S \tan\left(\frac{k_f d_f}{2}\right); \quad Z_{2f} = -i \frac{Z_f S}{\sin\left(\frac{k_f d_f}{2}\right)} , \tag{4}$$

where $Z_f = \left(c_{66}^{(f)} \rho_f\right)^{1/2}$ is the specific acoustic impedance of the mycelium film of thickness d_f . $c_{66}^{(f)}$ and ρ_f represent the shear elastic constant and density; $k_f = \omega / v_f$ the wave number and v_f the acoustic phase velocity in the film. The expressions of the phase velocity and specific mechanical impedance in the film are given by:

$$v_f = \sqrt{\frac{c_{66}^{(f)} + i\omega\eta_{66}^{(f)}}{\rho_f}}, \quad Z_f = \sqrt{\left(c_{66}^{(f)} + i\omega\eta_{66}^{(f)}\right)\rho_f} , \tag{5}$$

where η_{66}^{f} takes into account of the viscosity in the film. By applying once again the Kirchhoff's equations on the equivalent circuit of Fig. 2b, and following the procedure previously outlined, the theoretical dependency of the complex impedance of the loaded resonator was calculated, using the set of material constants of both quartz and electrodes, as evaluated in the previous step. Comparison of the theoretical and experimental data of the frequency dependence of the complex electric impedance, with use of the least-squares method, allowed us to evaluate the thickness of the mycelium film together with the complete set of material constants providing a successful match between theoretical and experimental data. Curves 1 in Fig. 3, show the frequency dependency of the real (a) and imaginary (b) parts of the impedance, for the resonator loaded by the electrodes and sensitive film, which better describe the experimental points.

The same procedure was used to evaluate the changes in thickness and material constants of the film, upon exposure to concentrations of different vapors and gases as well upon restoring in air. For each test run a first set of impedance data was taken; after that the volatile liquid was introduced into the chamber and a period of time of 10 min was waited for, so to allow all the liquid to be evaporated. At this point the second set of data was taken, repeated after 20 min before removing the resonator from the test chamber, and again in air after 10 and 30 min. At the end of each run the surface of the resonator was carefully cleaned and the complete restoring of the starting conditions checked. All the measurements have been carried out at a constant temperature of 26ºC. The changes in the material constants and thickness of the mycelium film in the presence of the different gaseous environments allowed us to estimate the sorption properties of the film.

Figure 3 shows, as an example, the frequency dependency of the complex electrical impedance of the resonator loaded by mycelium film before gas exposure (curve 1), after 10 and 20 min exposure to ammonia gas (curves 2 and 3), and finally after 10 and 30 min recovery in air (curves 4 and 5). The film was tested upon exposure to the following gases and vapors of volatile liquids: h-hexane, formaldehyde, acetone, acetic acid, ethyl-acetate, chloroform, hydrochloric acid, and ammonia.

3. Results and discussion

The values of the shear elastic constant, viscosity, mass density, and thickness of the mycelium films at all the stages of the investigation have been evaluated; it has been shown how the presence of vapors of volatile liquids leads to a decrease in the resonant frequency as well as an increase in the maximum value of the real part and a drop of the imaginary part of electrical impedance. Moreover it was experimentally demonstrated how the exploitation of specific technological methods of production of the film can provide resonators for which the resonant frequency and Q-factor are completely restored after a complete cycle of gas/vapor adsorption/desorption. The results so obtained show how some of the mycelium films analyzed are suitable for the development of ammonia, formaldehyde, and ethyl acetate sensors.

3.1. Sensitivity to ammonia

The film prepared from the *Lentinula edodes* F-249 monoculture mycelium grown in the synthetic medium fortified with 0.2 mg/l IAA for 14 days, and then extracted with ethanol (96%, *v/v*) can be used for ammonia sensing, as shown in Fig. 4a. Upon exposure to ammonia, the mass of the mycelium film increases sharply in about 10 min. By replacing the ammonia vapor with air, the film mass starts to revert to its initial value in about 10 min, and in the next 20 min the initial mass value is almost completely reached, as seen in Fig. 4 a. The film exposed to ammonia exhibited not only changes in the mass, but in the shear elastic modulus $c_{66}^{(f)}$ as well (Fig.4b) A decrease in $c_{66}^{(f)}$ was observed from $0.197 \cdot 10^{-8}$ to $0.106 \cdot 10^{-8}$ Pa after 10 m exposure to ammonia, being practically constant during the next 20 min. Upon exposure to pure air, the value of $0.211 \cdot 10^{-8}$ Pa was recovered during the first 10 min, then reaching the value of $0.204 \cdot 10^{-8}$ Pa after the next 20 min of exposure to air. On the basis of this result, one could conclude that the creation of a sensor coating sensitive to ammonia should implement the shiitake mycelium cultivation for 14 days in the liquid nutrient medium fortified with 0.2 mg/l of indolyl-3-acetic acid, and the ethanolic (96%, *v/v*) extraction of the mycelium so obtained. Further tests performed on the film have shown how it is not suitable to detect other vapors, such as: *n*-hexane, acetone, acetic acid, ethylacetate, chloroform, hydrochloric acid and formaldehyde.

3.2. Sensitivity to formaldehyde

Formaldehyde concentrations could be detected with the film obtained on the basis of the *Lentinula edodes* F-249 monoculture mycelium grown in the synthetic nutrient medium fortified with tryptamine at the concentration of 0.1 g/l for 14 days, and then extracted by the ethanol-H_2O (1:1, *v/v*) mixture. Exposure to gaseous formaldehyde gave rise to sharp changes in the values of both viscosity $\eta_{66}^{(f)}$ and elastic modulus $c_{66}^{(f)}$ of the film, as shown in Fig. 5 a and b, respectively. The interaction was shown to be reversible and after replacing formaldehyde with air, the initial values were restored. Analysis showed that, when placed in gaseous formaldehyde, the film exhibit an increase in the viscosity coefficient as high as more than 20 times, and a recovery time of about 30 min after the end of the exposure (Fig. 5 a). As to the elastic modulus (Fig. 5b), a decrease of 4 times was observed after 30 min exposure to gaseous formaldehyde and a complete recovery after 10 min exposure to pure air.

The reversibility of the film's properties, in the case of formaldehyde, was likely related to the film swelling in the presence of this gas, capable of escaping from the film after the end of the exposure. Biochemical reasons for such film behavior encourage further chemical studies.

Formaldehyde could also be detected using the films obtained on the basis of *Lentinula edodes* F-249 mycelium cultured in the synthetic nutrient medium fortified with indolyl-3-acetamide (0.1 g/l) for 14 days, and then extracted by the aqueous ethanol (50%, *v/v*). The viscosity coefficient and elastic module values of these films behave analogously to the parameters of mycelium grown in the presence of tryptamine additive (Fig. 5 c, d).

The analysis performed revealed again that the films described in this section were not adequate to detect the other vapors under test: *n*-hexane, acetone, ethylacetate, acetic acid, chloroform, hydrochloric acid and ammonia.

3.3. Sensitivity to ethylacetate

To detect the presence of ethylacetate, films obtained on the basis of the *Lentinula edodes* F-249 monoculture mycelium grown in the synthetic nutrient medium fortified with indolyl-3-acetamide at the concentration of 0.1 g/l for 14 days, and then extracted by the ethanol-H_2O (1:1, *v/v*) mixture, could be implemented. In this case the reversible change in the film's mass ρ_f (Fig. 6a) and viscosity coefficient $\eta_{66}^{(f)}$ (Fig. 6b) values was observed. It is noteworthy that the value of the viscosity coefficient appeared to be 7 times greater after exposure of the film to ethylacetate vaporous for 30 min (Fig. 6 b). After removing ethylacetate, the viscosity reached its initial value in 30 min. Regarding the changes in the film's mass, it was found that this value increased in 10 min after ethylacetate exposure, and decreased to the initial value in 30 min after exposure end.

Therefore the behavior of these films was analogous to that of the mycelium film grown in the presence of tryptamine additive (Fig. 5 c, d).

On considering the behavior of both the viscosity and elastic modulus under the effect of ethylacetate, it could be noted that it was analogous to that observed in the case of formaldehyde exposure. Obviously, ethylacetate vaporous also was capable of causing the film swelling. Thus, the different carbonylic compounds in respect to mycelial-extract-based film prepared with *Lentinula edodes* F-249 culture, essentially resembled each other in their effects.

Alike the previous cases, analysis revealed that this film was also inappropriate to detect other gases and/or vapors like *n*-hexane, formaldehyde, acetone, acetic acid, chloroform, hydrochloric acid, and ammonia.

4. Conclusion

The analysis carried out in the present work has shown how mycelium films may be successfully used as a sensitive layer for electro-acoustic chemical sensor applications, suitable to detect the presence of gases and vapors in environment, detrimental to the human health. This is connected with the fact that mycelium films are very prospective novel gas sensitive material, whose properties are recovered after end of the gas or vapor action. It has been found that for ammonia sensor, the film prepared from the *Lentinula edodes* F-249 monoculture mycelium grown in the synthetic medium fortified with 0.2 mg/l IAA for 14 days, and then extracted with ethanol may be used. As for formaldehyde, its presence can be detected with the aid of the film obtained on the basis of the *Lentinula edodes* F-249 monoculture mycelium grown in the synthetic nutrient medium fortified with tryptamine at the concentration of 10^{-1} g/l for 14 days, and then extracted by the ethanol-H_2O (1:1, *v/v*) mixture or with the aid of the films obtained on the basis of *Lentinula edodes* F-249 mycelium cultured in the synthetic nutrient medium fortified with indolyl-3-acetamide (0.1 g/l) for 14 days, and then extracted by the aqueous ethanol (50%, *v/v*). To discover the presence of ethylacetate, the films obtained on the basis of the *Lentinula edodes* F-249 monoculture mycelium grown in the synthetic nutrient medium fortified with indolyl-3-acetamide at the concentration of 0.1 g/l for 14 days, and then extracted by the ethanol-H_2O (1:1, *v/v*) mixture, may be implemented. The results so obtained show the possibility to develop multisensory analyzers of gases or vapors mixtures on the mycelium films basis [20], where the composition of the mixture can be evaluated by the analyzing the set of changes in the material data density, elastic constant and viscosity coefficient.

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Figure captions

Fig.1 – Experimental setup. Front (a) and top (b) view of the resonator coated with the mycelium film. Test chamber (c).

Fig.2 – Mason's equivalent circuits for the unloaded resonator (a) and for the resonator loaded with the mycelium film (b).

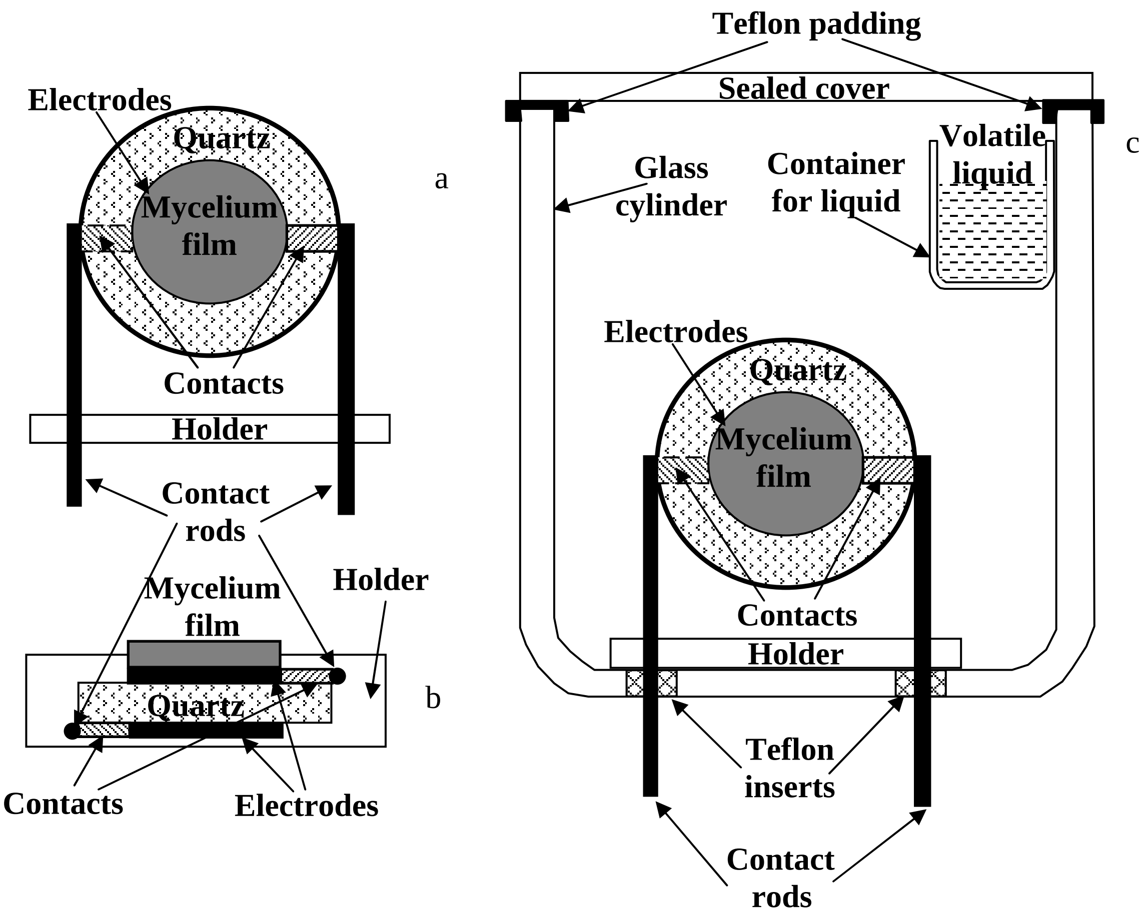
Fig.3 - Frequency dependency of the real and imaginary parts of the electrical impedance for the resonator loaded by mycelium film. Before gas exposure (curve 1), after 10 and 30 m after gas exposure (curves 2 and 3), after 10 and 30 m recovery in air (curves 4 and 5). The data are referred to ammonia gas.

Fig.4. Time dependency of the relative change in mass (a) and absolute change in the elastic modulus $c_{66}^{(f)}$ (b) of the mycelial film produced by ammonia. *Lentinula edodes* F-249 mycelium, 14-days-aged, synthetic nutrient medium fortified with indolyl-3-acetic acid, 0.2 mg/l, extracting agent ethanol (96%, *v/v*).

Fig.5. Time dependency of the absolute changes in the viscosity coefficient (a, c) and the elastic module (b, d) of mycelial film under study caused by formaldehyde. *Lentinula edodes* F-249 mycelium, 14-days-aged. Extracting agent is aqueous ethanol (50%, *v/v*). Synthetic nutrient medium fortified with: tryptamine, 0.1 g/l (a, b), indolyl-3-acetamide, 0.1 g/l (c, d)

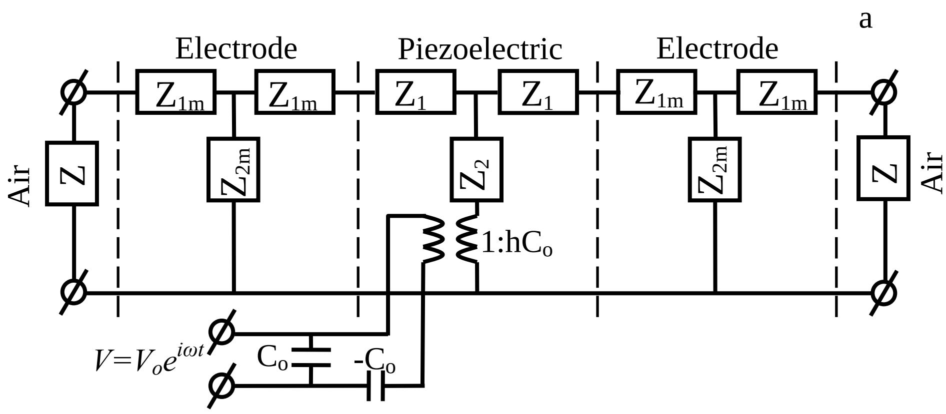
Fig.6. Time dependencies of the relative change in mass (a) and the absolute change in the viscosity coefficient (b) of mycelial film under study caused by ethylacetate. *Lentinula edodes* F-249 mycelium, 14-days-aged, synthetic nutrient medium fortified with indolyl-3-acetamide, 0.1 g/l. Extracting agent is aqueous ethanol (50%, *v/v*).

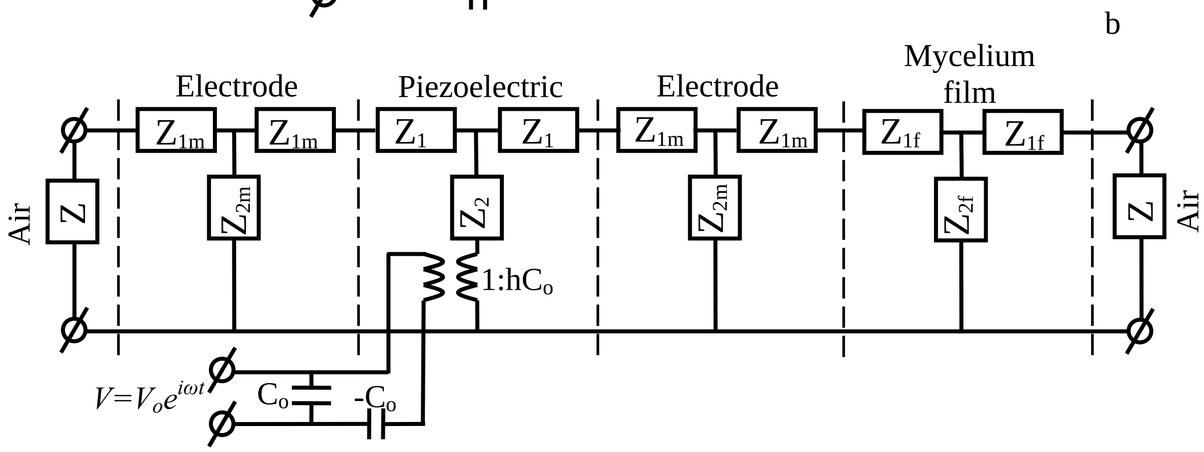
Figure(s)



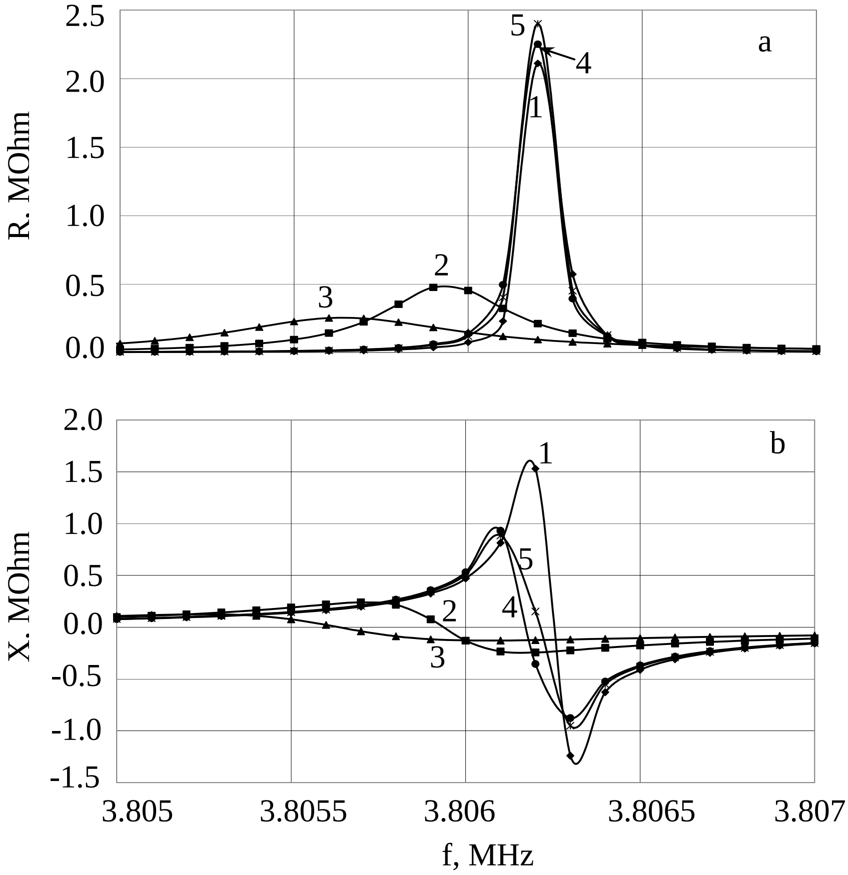
Electrodes
Quartz
Mycelium film
Contacts
Holder
Contact rods
a
Mycelium film
Holder
Quartz
Contacts
Electrodes
b
Teflon padding
Sealed cover
Volatile liquid
c
Glass cylinder
Container for liquid
Electrodes
Quartz
Mycelium film
Contacts
Holder
Teflon inserts
Contact rods

Figure(s)


a
Electrode
Piezoelectric
Electrode
Z1m
Z1m
Z1
Z1
Z1m
Z1m
Air
Z
Z2m
Z2
Z2m
Z
Air
1:hCo
V=Voeiωt
Co
-Co

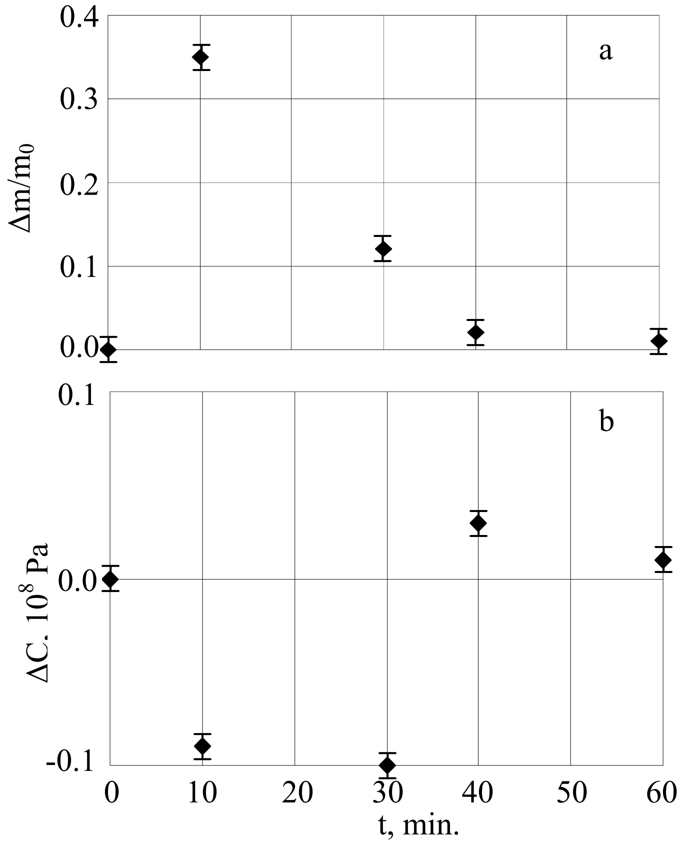

b
Mycelium film
Electrode
Piezoelectric
Electrode
Z1m
Z1m
Z1
Z1
Z1m
Z1m
Z1f
Z1f
Air
Z
Z2m
Z2
Z2m
Z2f
Z
Air
1:hCo
V=Voeiωt
Co
-Co

Figure(s)

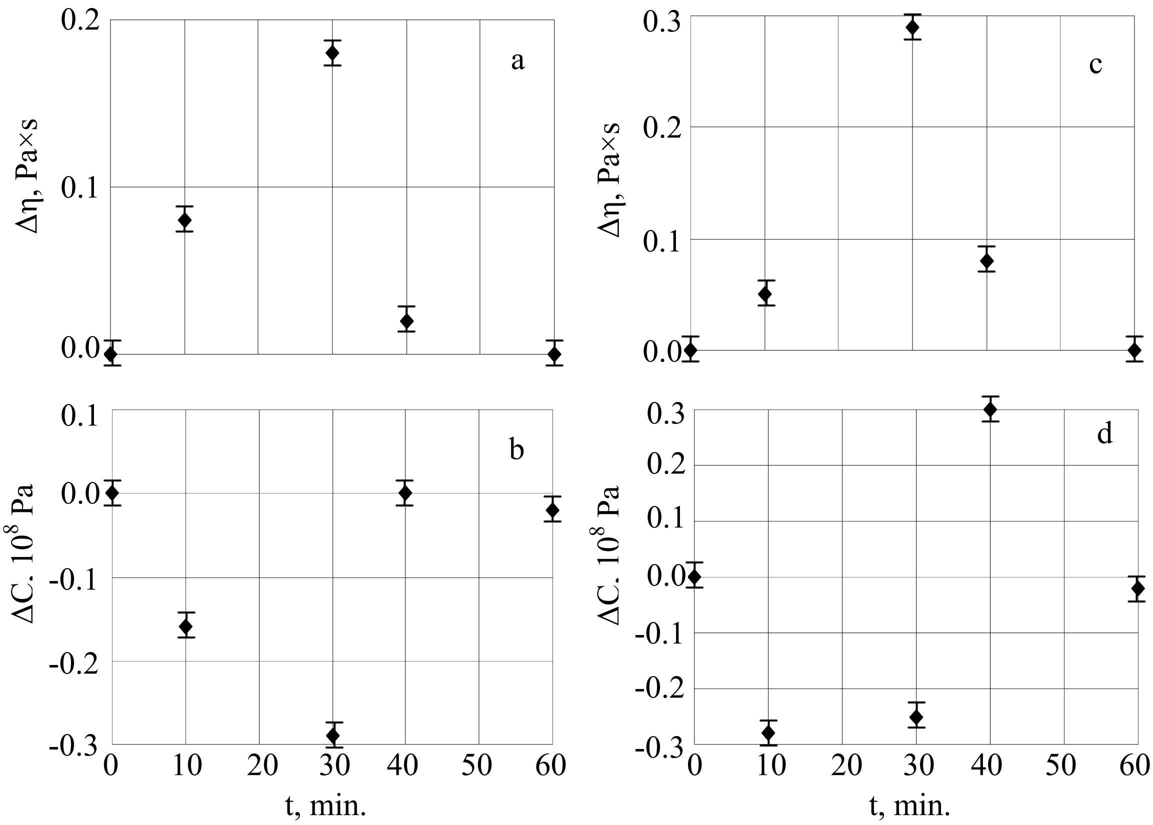


2.5
2.0
1.5
1.0
0.5
0.0
R. MOhm
5
4
1
2
3
a
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
X. MOhm
1
5
4
2
3
b
3.805
3.8055
3.806
3.8065
3.807
f, MHz

Figure(s)



Figure(s)



0.2
0.1
0.0
Δη, Pa×s
a
0.3
0.2
0.1
0.0
Δη, Pa×s
c
0.1
0.0
-0.1
-0.2
-0.3
ΔC. 10^8 Pa
b
0 10 20 30 40 50 60
t, min.
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
ΔC. 10^8 Pa
d
0 10 20 30 40 50 60
t, min.

Figure(s)