SYMBIO Biomonitoring System

Content

1. About company

2. Controlling water quality at water intakes

- 2.1. Introduction
- 2.2. The idea of bioindication
- 2.3. General information about SYMBIO system

3. SYMBIO Biomonitoring System – operational schematic

4. The structure of SYMBIO Biomonitoring System

- 4.1. Tank
- 4.2. Controller
- 4.3. Computer software
- 4.4. Signaling a sudden change in water quality alarms and warnings

5. Optional equipment for SYMBIO system

6. Biological material

- 6.1. Living conditions in the natural environment
- 6.2. Mussels functioning in the SYMBIO system

7. SYMBIO system servicing

- 7.1. Acquisition of biological material
- 7.2. Acclimatization
- 7.3. Placing mussels in the system and their replacement

8. Certificates, honorable mentions and references



1. About company

PROTE Technologies for our Environment has been operating in the field of environmental protection in Poland since 1995. Initially as a company lead by a natural person, then as a limited liability company, PROTE renders services at the highest level, comprehensively carrying out projects related with environmental protection. Full satisfaction of our Clients is our primary goal on the way to build PROTE brand as a proven and reliable trade partner in projects related with environmental protection. We have successfully carried out nearly 300 projects and proved to be reliable, trustworthy and dedicated, this way obtaining experience necessary to perform subsequent contracts. Introducing modern technologies together with comprehensive service we assist in resolving environmental problems and preventing degradation of the environment. Our team consists of specialists in this field, people combining knowledge and skills. Thanks to them PROTE grows stronger and develops, in ecological solutions, even in those which are far from traditional ones. Our activity is marked by an individual character of designing and completion with optimization of the tools and technologies used.

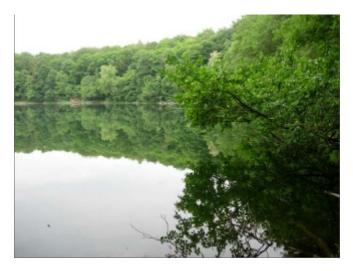
PROTE has implemented and maintains quality, environment and health and safety management systems according to standards: ISO 9001-2000, ISO 14001:2004 and OHSAS 18001:1999 issued by one of the world's oldest certification centers BSI Management.

In the year 2007 we received a certificate confirming the compliance of our actions with the requirements of NATO AQAP 2110:2009 standards. Quality is the manifestation of active involvement of all company's employees in the functioning and improvement of the implemented systems. The proof of our achievements regarding the quality of our services are the numerous awards, certificates and references, including: four Gold Medals of the Poznan International Fair awarded during the International Ecological Fair POLEKO in 2001, 2004, 2010, 2012, trade mark "Greater Poland's Quality" in 2002, title of the laureate of the ProEco Award from the European Institute of Quality in 2004, as well as the EuroEco Award in 2006 (the first company in Poland to be awarded this title), dozens of Letters of Satisfaction from our Clients. In 2007 we joined the group of laureates of the 10th edition of the "Company Fair Play" program. Moreover, we are among those few companies which have 4 awards and 1 honorable mention of the Polish Promotional Emblem TERAZ POLSKA. In 2007 we received the TERAZ POLSKA Award in the best service category – soil and water environment reclamation with the use of TIB Intensive Bioremediation Technology, in 2008, in the best product category – SYMBIO Biomonitoring System, in 2011 – in the best service category for the comprehensive service of lakes reclamation with the use of the innovative PROTE-fos method, and in 2013 for the SeaQuest comprehensive service of water supply systems cleaning counteracting secondary contamination of the distributed water. We believe that the most reliable indicator of our partnership approach in the field of environmental protection is verification of the achievements presented above by establishing cooperation, to which we cordially invite you.

2. Controlling water quality at water intakes

2.1 Introduction

It is not said by a coincidence that water is the source of life, as it is the main part of each living cell, it also constitutes the environment for 80% of fauna and flora species, and covers about 70% of the area of our Planet. The amount of freshwater constitutes only 3% of all the water present in the biosphere, and it is mostly held in icebergs –this should make us think how precious its resources are. Poland's water resources on the other hand are marked by seasonal changes and uneven distribution over the territory of our country. Poland is among countries which have rather small surface water resources. Annually per capita it is about 1600m³ of water, while in other European countries it is 3-4 times more. The data cited above show how important and precious fresh water is, and how important it is to protect its natural resources and safely distribute them to their recipients.



Quality controls carried out by Waterworks and Sanitary Inspectors are to guarantee that the water distributed to recipients conforms to standards described in regulations. The analyses are carried out with a limited frequency and scope which does not guarantee that the water treated in one moment does not contain toxic substances or has not been contaminated. Recently more and more frequent are water source contamination and other events which might lead to limiting water distribution to recipients. Water intakes are prone to contamination with chemical compounds such as nitrites, pesticides, heavy metals etc. Especially surface waters are prone to taking up all sorts of contaminants, which appear in water as a result of surface runoff, discharge of untreated sewage, illegal discharges of contaminants and industrial malfunctions or the use of chemicals in farming. In view of such hazards it is becoming very important not only to lead a panoramic monitoring of water but also a continuous control of the quality of water at water intakes.

The solution which we propose enables us to guarantee such a protection thanks to which waterworks are sure that the water undergoing treatment and distributed to recipients does not contain hazardous toxic substances. Here we have in mind a system that is based on the idea of bioindication, which has accompanied humans for a very long time. In former times people did not know methods of analysis and control of the state of water intakes, but they tried to use horses and

fish as bioindicators helping to detect possible pollution of water which was to be consumed by humans. Similarly canaries were used in mining to inform about the hazard of poisoning with harmful gases. These methods might have been primitive but they were so effective that they saved peoples' lives many times, although it was not known what kind of substance and in what quantities polluted the air or the water. Our system is based on a similar idea, and it has been installed on water intakes to continuously control them. We talk here about the SYMBIO Biomonitoring System uses as living indicators of environmental pollution fresh water mussels. These organisms thanks to their sensitivity to any possible toxic compounds appearing in water react with a sudden and total shell closing. Mussels have every characteristics of bioindicators and when compared with other such organisms they have many positive features. That is why these organisms (also considering the economic factor) were selected as living indicators for the SYMBIO Biomonitoring System, and they are a continuation of used in former times bioindication methods in a modern setting. Nowadays it is possible to electronically control the behavior of bioindicators (mussels), to archive the collected data, on-line functioning of the system, or remote access to the system.

2.2 The idea of bioindication

Bioindication is a method which uses living organisms as detectors of environmental pollution. One of bioindication streams (environmental) analyzes the influence of toxic substances on the environment, the other is related with the human. Bioindicators can be animal or plant species which have a very limited tolerance to certain factors (the so called stenotypical species).

Bioindication is used, among other things, for water quality assessment. Developing a system for controlling water quality at water intakes is especially important for water supply systems which draw from surface water sources. One of main characteristics of surface water sources is the fact that they tend to receive all kinds of pollutants resulting from surface runoff, dumping untreated or not sufficiently treated sewage, leachates from landfills, industrial facilities' malfunctions, chemicalization of agriculture, etc. Water drawn from lakes, storage reservoirs or rivers, despite exclusion zones, can be contaminated as a result of ecological disasters, breakdowns or terrorist attacks. An improper quality of water in lakes is often related to biological processes inside a particular water reservoir (such as algal blooms), as well as progressive development of civilization and disrespect towards basic rules of environmental protection, which leads to a rapid progress in lakes eutrophication and worsening of water quality.

In water supply systems which draw water from rivers situations of emergency incidental water contamination are most likely to occur. Water intakes' exclusion zones include only a particular part of a river, above the intake, and the pollution in the flowing water can easily migrate. Ground waters, as they are naturally isolated from the ground surface, are much less prone to contamination. In the recent years, however, e.g. pesticide-related pollution of these waters has increased.

The assessment of water contamination level by means of bioindication methods can be carried out through short-term inbreeding, called biotests, or through long-term observation and recording the behavior of aquatic organisms on a continuous basis – biomonitoring. Observed behavioral changes of bioindicators are related to stress resulting from adverse or harmful external factors. The criteria for selecting organisms which will optimally function in a biomonitoring system are strictly defined:

• Their reaction to changes in the environment must be quick and reliable,

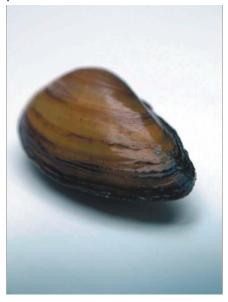
- Their reactions should be unambiguous and easy to interpret,
- Maintaining bioindicators in laboratory conditions shouldn't be costly or effort-consuming,
- The habitat and behavior of these organisms must allow us to monitor them automatically and on a continuous basis.

Bioindication enables us to determine total toxicity of all harmful substances which, in many cases, work synergistically. Hence bioindicative method provides assessment of total toxicity of the analyzed system and is a perfect complement to chemical analyses periodically carried out by laboratories monitoring water. This method also enables us to increase the frequency of performed observations, often enough up to real-time observations (on-line).

2.3. General information about the SYMBIO Biomonitoring System

SYMBIO Biomonitoring System is used for controlling the quality of water at water intakes on a continuous basis. The system incorporates fresh water mussels, whose natural reaction to a sudden change of environmental parameters is shell closing. Mussels applied in the SYMBIO system (Unio tumidus – a local species) meet all the criteria for indicator organisms: they quickly and unambiguously react to changes in the environment, their reaction (shell closing) is easy to observe and record, and their living conditions and lifestyle enable us to monitor their behavior in laboratory conditions on a continuous basis.

Mussels' reaction does not provide us with the information about what in particular and in what quantities has gotten into the water, but a sudden shell closing signalizes a change in water parameters – which they recognize as harmful and hazardous.



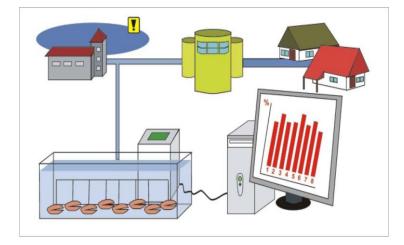
SYMBIO usage does not require a qualified personnel and merely comes down to reacting in alarm situations which are acoustically and visually signalized. The role of the system is to generate an alarm signal in case of an incidental water contamination. Hence the system is a perfect complement to periodical laboratory analyses of water. The method enables us to detect a sudden change in water quality, resulting from a toxic substance occurring in the water. Information on water quality is archived and delivered every single second. PROTE as the system's manufacturer designed the

equipment to be functional, infallible and having low operation cost. Mussels placed in a system are replaced every three months in order to assure proper sensitivity of indicator organisms and ensure that during this period the system is maintenance-free. These works are performed within the service provided by PROTE company. Our experience in cooperation with Polish waterworks has enabled us to adapt SYMBIO system to the real working conditions, at the same time meeting the high requirements for modern security systems. The evaluation of the system performed by an independent laboratory confirmed its CE marking compliance. What is more the system was awarded a Gold Medal of the International Ecological Fair POLEKO 2004 as well as the Award of the President of the Republic of Poland, the TERAZ POLSKA promotional emblem, which confirms the system's infallibility, safety and functionality, as well as its high-quality manufacturing.

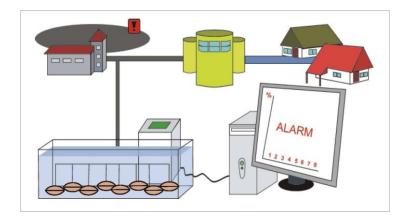
SYMBIO systems installed at water intakes undoubtedly contribute to an increase in the safety of water recipients, and enables the managers of water distribution companies to be sure that the water undergoing treatment is safe for consumers' health. The economical aspect of using a system also plays an important part and the system has low operational costs. If we take into consideration the average water price in Poland, daily costs of maintaining the system equals, more or less, a revenue from selling 10 m³ of water. Such low operational costs are acceptable even for small water distribution companies, especially that during one year water is sampled 31 500 000 times.

3. Schematic showing how the SYMBIO Biomonitoring System works

Monitoring water quality at water intake by means of SYMBIO system - schematic



Mussels reaction to water contamination - alarm situation



4. The construction of a SYMBIO Biomonitoring System

4.1 Tank

A SYMBIO tank, in which eight mussels are place at the same time, is made of stainless steel. Its construction eliminates or minimizes the influence of external factors which could have a bad effect on mussels' activity. The tank provides shade and isolation from noise, and, to a great extent, it cushions small vibrations of the ground. An aerating filter installed inside the tank guarantees proper oxygenation of water. To each mussel a measuring probe is conveyed that works together with a magnet attached to the mussel's shell registering the mussels' activity.



4.2 Controller

The controller is integrated with the system's tank. The role of the controller is to process data and send it to the computer as well as take over the function of generating alarms in case of the computer's or software's malfunction (imperative in regard to the controller). On the controller's operating terminal the current openness level of each of the mussel specimens placed in the SYMBIO system can be read.

The controller enables us to connect additional sensors, such as temperature sensor or the sensor responsible for sending data to the computer. Each time after the mussels are installed in the system the system's calibration is performed. During a calibration two conventional extremes (minimal and

maximal shell's openness level) are set to designate a working corridor for each track. Next, during the following few days, these hand settings are verified and provided with more details by means of autocalibration.



4.3. Computer – software

The role of the computer program is to visualize and archive data as well as to create and save reports. It enables us to perform a current assessment of the system's work and to track mussels' activity over a period of time in the past.

Visualization of data in form of bar charts shows the current percentage of each of the eight mussels' openness level (water is sampled every single second)., while the line charts enable us to track mussels' activity over a period of time in the past.

PROTE's experience in the cooperation with water distribution companies as well as other institutions, where SYMBIO systems are installed, has enabled us to make numerous improvements in the system's software. The introduced changes allow for a better control of the system's work and ensure its infallibility and maintenance-free operation. In addition the improved system's software allows for remote connection with the system through the Internet, and in cases when service work are performed by PROTE. The software also allows for sending e-mail messages (or sms in form of e-mails) including information about events that have taken place in the system. Access rights to the system are divided (either data tracking or access to the system's settings), which protects the system from any interference of third parties into the system and introducing changes in the program's settings, which could affect its work.

SYMBIO system is presented on the computer's screen by means of the following windows:

- main menu
- bar charts showing the current openness level of each mussel the current activity
- line charts showing the openness level of each mussel historical data related to mussels' activity
- list of events.

Description of SYMBIO system's software

The main characteristic feature of the SYMBIO system's software is its two-module architecture. The software consists of SYMBIO Server and SYMBIO Client, which allows for a high level of system's security protecting it from unwanted interference of third parties as well as for a higher degree of autonomousness of the system's work.

SYMBIO Server software

- it is the imperative part of the software comprising the core of the software,
- it is responsible for collecting data sent from the system's controller,
- it is responsible for regularly sending data to the Base in the computer in PROTE's headquarters, which eliminates the former need to record reports directly from the Client's system for their further analysis,
- it is launched automatically when the computer is turned on,
- it is maintenance-free and its work on the computer is encrypted in order to eliminate the possibility of its accidental turning off,
- it allows for remote connection with the system by means of a computer network with an Internet access,
- it allows for sending e-mails (or SMS in form of e-mails) to inform about events which have taken place in the system,
- it enables us to divide access rights to the SYMBIO software,
- it can serve up to 5 remote connections from SYMBIO Client programs from different locations with an Internet access in order to observe the current state of the system and current mussels' activity.

SYMBIO Client software

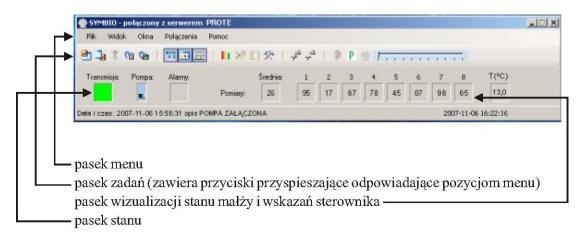
This part of SYMBIO system is a type of browser which allows for the following:

- observation of the current state of the system and current mussels' activity on a local computer (in the place where SYMBIO Server works as well),
- browsing archive data collected on the local computer,
- reacting to warnings and alarms,
- connecting with remote systems in order to observe the current state of the system as well as the current mussels' activity.

Main window

The main window of the browser (SYMBIO Client) is a central point of the application, from which it is possible to switch into any other window of the application. It contains the menu and taskbar, it enables the user to implement functions of the program and visualizations of the current state of the controller.

The information shown in the menu enable the user to generally assess the work of the system (for example signalization of the lack of transmission, alarms). Function keys are used to implement an appropriate function or enable the user to display a window which shows detailed data.

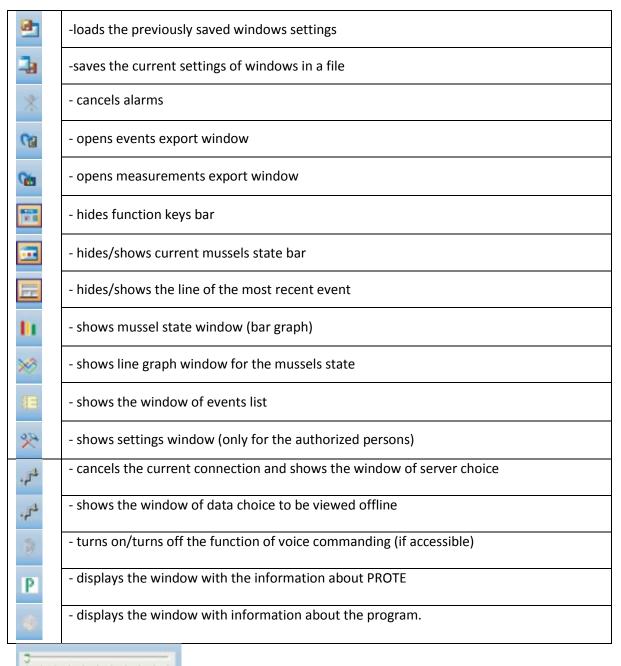


Menu bar

- \circ File
 - Window settings
 - ✓ Load it loads the settings of program windows saved previously
 - ✓ Save it saves the current window settings to a file
 - Cancelling the alarm it cancels alarms and warnings.
 - Export

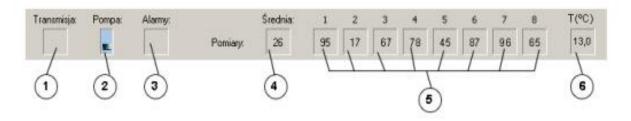
- ✓ Of Events it displays the window of events export, from a given period, which took place in the system, to a text file
- ✓ Of Alarms it displays the window of measurements export from a given period, to a text file.
- Finish it finishes the work of the program.
- o View
- Function keys hides/shows the BAR of function keys in the main window
- Current state of mussels hides/shows the BAR of mussels visualization, state of the pump, indications of the controller (warnings, alarms)
- Last event hides/shows the BAR displaying the recent event that took place in the system
- o Windows
 - Bar graph shows the WINDOW OF THE CURRENT STATE OF MUSSELS (current activity)
 - Line graph shows the WINDOW OF HISTORY pertaining to mussel activity
 - List of events shows the WINDOW OF THE LIST OF EVENTS, which took place in the system within a certain period of time
 - Settings shows the WINDOW OF SETTINGS for the system (in view of the key importance of the settings for the proper work of the system this window is only accessible for the authorized persons).
- Connections
 - New it disconnects with the SYMBIO Server and displays CHOICE WINDOW OF THE SERVER.
 - Working offline displays WINDOW OF DATA CHOICE to be viewed offline (without the necessity to be connected with the SYMBIO Server).
- o Help
 - PROTE –information about the company.
 - SYMBIO information about the program.

Taskbar – icon descriptions:



transparency of the window.

The bar of mussels state and controller indications visualization



- 1. Transmission state field if the transmission state is proper this field flashes in green, no transmission is signalized by flashing in red.
- 2. The field of pump/filter inside the flow-through tank state this field is presented in a form of a moving element. Its movement presents a working pump, in the situation when the movement is stopped and the field has a red frame, it signalizes that the pump is switched off. Turning the pump on again can be done by clicking this field (there is no, however, a possibility to turn the pump off this way first because it is an undesirable situation, second, because someone might do it accidentally).
- 3. Program alarm field in case of no alarms the field has the color of the background, in case of alarms it takes the color according to the type of alarm (red or yellow) or a warning (green or blue).
- 4. The field of the average and controller alarms the field contains the average value of the active channels (mussels) and in case of controller alarms it signalizes the average alarm in red, and the quantity alarm in yellow. It is important to know that controller alarms are subordinate towards program alarms.
- 5. The fields of each mussel state each of these fields displays the current percentage value of the level of openness of the given mussel for the proper controller channel (marked with a number above the field from 1 to 8). This field signalizes a mussel entering the state if observation for weak specimen this state is signalized by orange color.
- The field of indications and alarms of the temperature probe (optionally) this field displays the current temperature and in case of exceeding the set border values it turns red.

State bar

| Data i czas : 2007-08-28 11:21:47 opis ZMIANA WEJŚĆ | 2007-08-28 11:21:49 | | |
|-----------------------------------------------------|---------------------|--|--|
| Ū | 2 | | |

- 1. Most recent event label.
- 2. SYMBIO Server connection time label.

Bar graphs presenting the current state of mussels shells openness

This window contains bar graphs, illustrating percentage level of openness of each mussel, (marked from M01 to M08) as well as the bar illustrating the average of all installed specimen.

The update of data transmitted from the controller to the computer is done every single second.



Parts of the graph window

- 1. The head of mussel openness state
- 2. The complement of the mussel openness state
- 3. The mussel openness state
- 4. The complement of the bar of mussel openness threshold
- 5. The openness threshold bar
- 6. The percentage openness level
- 7. The key for the current window saving of the graphic file in a chosen catalogue
- 8. The configuration key of the bar graph window

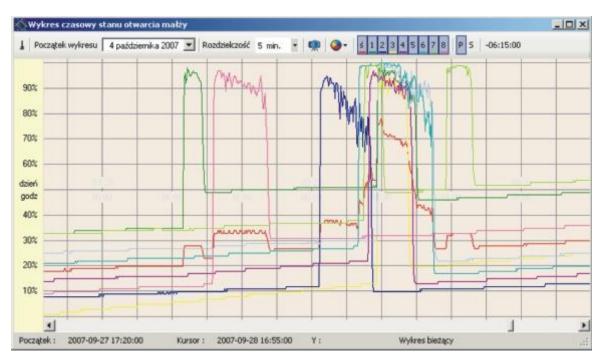
Line graphs presenting mussel openness state in the function of time for a chosen period

Each specimen is presented by one color in the graph. The assignment of colors to the mussels can be checked in the head of the window. One color is reserved for the average graph.

In the ordinate we can read the percentage openness level of a mussel, and in the abscissae we can read the day and time. Fluctuations upwards and downwards from the horizontal line on the graph illustrate mussels activity (filtrating water).

The display of the graphs can be done in the following modes:

• Current – in the window there is data read in the given moment, after each period of probing, the graph is moved to the left,



• Historical – in the window here is archive data.

In the head of the window there is a bar with function keys, which allow us to do the following operations:

- Choosing a date of the beginning of the graph by clicking the arrow displays the calendar in which we may choose a day of which we would like to display data; if we choose the current day, then after each registration of the measurements, the graph is moved to the left, while at the end of the graph there are the newly registered measurements.
- Choosing the probing period (resolution the level of averaging the displayed data),
- Saving the window in a file
- Turning on, turning off and changing of colors of the graph lines for each measurement channel in the head of the window there are colorful keys used for controlling the process of displaying the given measurement circuit (turning off, turning on, color change). Keys from 1 to 8 show the different measurement circuits (mussels), the key Ś means the average mussel openness level in the population.

In the bottom panel, when we slide the slider to the left, we can see the graph in a historical time. The beginning displays the date and time of the beginning of the graph. In the cursor time (day and time) is defined. In the Y position there is a percentage level of openness of mussels adequate to the position of the cursor. With the use of the cursor you can view on a given graph the % openness level of a mussel (position Y), in a chosen period of time.

List of events

In this window there is information presented registered in the events report. Each line contains the following fields:

- Data and time of occurring
- Event description and time of occurrence
- Arguments depending on a type of the event: circuit number, value of change, etc.

The events can be displayed in the current or historical mode.

| Wybór czasu | 11 lipca | 2007 | 💌 🧖 Lista wg kalendarza | | | |
|----------------|------------|----------|-------------------------------|-------|----------|----------|
| Тур | Data | Czas | Opia | Kanał | Dane (1) | Dane [2] |
| ostrzeżenie | 2007-07-11 | 18:33:00 | PRZEKROCZONY PRÓG WARTOŚCI | 0 | | |
| A ostrzeżenie | 2007-07-11 | 18:32:41 | PRZEKROCZONY PRÓG WARTOŚCI | 0 | | |
| A ostrzeżenie | 2007-07-11 | 18:32:29 | PRZEKROCZONY PRÓG WARTOŚCI | 0 | | |
| Informacja | 2007-07-11 | 17:30:24 | POMPA ZAŁĄCZONA | | | |
| Informacja | 2007-07-11 | 17:30:24 | ROZPOCZĘCIE AUTOKALIBRACJI S | | | |
| Dinformacja | 2007-07-11 | 17:30:15 | START PROGRAMU | | | |
| ostrzeżenie | 2007-07-11 | 17:26:13 | START KALIBRACJI KANAŁU | 7 | | |
| A ostrzeżenie | 2007-07-11 | 17:22:22 | ZAKOŃCZENIE KALIBRACJI KANAŁU | 6 | | |
| alarm | 2007-07-11 | 17:21:51 | POWRÓT TRANSMISJI | | | |
| alarm | 2007-07-11 | 17:21:50 | ZANIK TRANSMISJI | | | |
| ostrzeżenie | 2007-07-11 | 17:20:54 | START KALIBRACJI KANAŁU | 6 | | |
| ostrzeżenie | 2007-07-11 | 17:20:41 | ZAKOŃCZENIE KALIBRACJI KANAŁU | 8 | | |
| 1 alarm | 2007-07-11 | 17:20:14 | USUNIĘTY ALARM STEROWNIKA S | | | |
| ostrzeżenie | 2007-07-11 | 17:18:55 | START KALIBRACJI KANAŁU | 8 | | |
| ostrzeżenie | 2007-07-11 | 17:18:43 | ZAKOŃCZENIE KALIBRACJI KANAŁU | 7 | | |
| S informacja | 2007-07-11 | 17:17:02 | ZMIANA CZASU DLA ILOŚCI | | 7 | 600 |
| 2 informacja | 2007-07-11 | 17:16:48 | ZMIANA WSKAŹNIKA ILOŚCI | | 10 | 75 |
| 9 informacija | 2007-07-11 | 17:15:57 | ZMIANA PROGU | 2 | 89 | 2 |
| 2 informacja | 2007-07-11 | 17:15:36 | ZMIANA PROGU | 1 | 92 | 2 |
| ostrzeżenie | 2007-07-11 | 17:15:35 | START KALIBRACJI KANAŁU | 7 | | |
| ostrzeżenie | 2007-07-11 | 17:14:04 | PRZEKROCZONY PRÓG WARTOŚCI | 6 | | |
| 👖 alarm | 2007-07-11 | 17:14:00 | ALARM STEROW/NIKA SBM | | | |
| ejoermotni 🔮 | 2007-07-11 | 17:14:00 | POMPA WYŁĄCZONA | | | |
| Pintormacja | 2007-07-11 | 17:14:00 | ROZPOCZĘCIE AUTOKALIBRACJI S | | | |
| 9 informacija | 2007-07-11 | 17:13:53 | START PROGRAMU | | | |
| a informacija | 2007-07-11 | 16:21:20 | POMPA ZAŁĄCZONA | | | |
| () informacija | 2007-07-11 | 16:21:20 | ROZPOCZĘCIE AUTOKALIBRACJI S | | | |
| 9 informacja | 2007-07-11 | 16:21:14 | START PROGRAMU | | | |

4.4 Signalization of a sudden change in water quality – alarm

In the conditions of normal functioning each mussel biorhythm can be divided into periods of higher and lower activity. In times of lower mussel activity, the shell might be partially or even completely closed for a period of a dozen or so hours. Shell closing even up to a few % or its gradual closing up to zero does not have to be a manifestation of stress. Only a sudden closing, and what is important, if is observed as a group reaction can be understood as a reaction to stress. At the moment of occurring a sudden change in water quality the mussels rapidly close, and the system generates an alarm (red or yellow). The alarm is signaled on the monitor of the computer, acoustically by the speakers and through a signal lamp.



The graph of mussel openness state after a toxic substance appeared in the water.



Signal lamp during an alarm.

In the SYMBIO system there are two program alarms and two program warnings.

The first two are to alarm in the situation of a sudden water contamination, the other two (warnings) are to eliminate the possibility to take into consideration events which have nothing to do with a toxic substance appearing in the water. The system enables us to early detect a weakening specimen and eliminate it from the group of mussels monitoring the water (until the arrival of the service).

Such a specimen is not calculated into the whole population or alarm group, and therefore it has no influence on alarm situations.

| Alarms and | SIGNALIZATION | | | | |
|---------------------|--------------------------------------|-------------------------------------|--------------|--------------|--|
| warnings Program | In the main window of SYMBIO program | | Sound | Lamp | |
| | Field: alarms | Flashing key of canceling alarms | | | |
| Red | Red | ✓ | ~ | ✓ | |
| Yellow | Yellow | ✓ | ~ | ✓ | |
| Green | Green | ✓ | | ✓ | |
| Blue | Blue | ✓ | | ✓ | |
| SBM controller | Field: average | | | | |
| Average | Yellow | ✓ | ✓ | ✓ *1 | |
| Quantity | Red | ✓ | ~ | ✓ *1 | |
| Transmission | Field: transmission | | | | |
| Transmission | Red | ✓ | ✓ | | |
| Temperature | Field: T | | | | |
| temperature | red | \checkmark | \checkmark | \checkmark | |

Types of alarms and their identification

*1 If only the controller is working and an alarm is generated, it will be signalized only by lamp. In other cases (proper computer and software work) the signalization will be seen only as it is shown in the table.

As an additional protection of the continuity of water monitoring it is possible for the controller to work independently in case of computer or software malfunction.

Alarm

\land Warning

Program alarms and warnings

Red (I degree) and yellow (II degree) signalizes a sudden change in water quality

- Red alarm rapid closing of a large group of mussels
- Yellow alarm closing of a large group of mussels over a longer time

Green and blue (group warning) informs about the closing of a group of mussels as a result of their natural activity. Such an event can take place in a situation when a group of mussels at the same time is in a similar stage of its biorhythm.

Controller SBM Alarm

Takes over the alarm functions in a situation of a possible malfunction of the software or/and computer. Controller alarm means a sudden change in water quality (red or yellow alarm).

Transmission Alarm

Transmission alarm means that there has been an error in sending data between the controller and the SYMBIO system computer. If such an alarm occurs PROTE company should be informed, we will diagnose and eliminate the technical problem being the cause of the alarm.

Temperature Alarm

It means that the temperature threshold was exceeded. You should measure the temperature with a thermometer and check the work of devices responsible for keeping the right temperature. The right temperature should be restored in the tank.

5. Optional SYMBIO system equipment

The presence of excessive amounts of suspended solids in the water intake flowing into the SYMBIO tank can cause the mussels siphons to clog. In view of this fact, depending on water quality in the water intake optionally filters are used. Water temperature, optimal for mussels is between 6-17% degree C. That is why the system installation is carried out in such a way that optimal temperature conditions are set for the mussels. A very important thing is water oxygenation (the amount of oxygen cannot drop below 4 mg/L).

The proper physical preparation of water for monitoring by mussels, with regard to the above mentioned parameters guarantees a proper functioning of bioindicators and reliability of the system.

In case of surface water intakes (especially when the water is taken from a river) it is
probable that periodically an increase of turbidity will occur. In order to sieve these
suspended solids from the water before the tank an exterior filter is installed.



 In case of underground water intakes and excessive amounts of iron in the water, which as a result of additional oxygenation can precipitate in form of suspended solids before SYMBIO tank a small iron filter is installed.



 In the situation of periodical changes in the temperature of the water (which might be the case in surface intakes) in order to guarantee proper temperature conditions before the SYMBIO tank devices which regulate the temperature of the water are installed.

6. Biological material

6.1. Living conditions in the natural environment

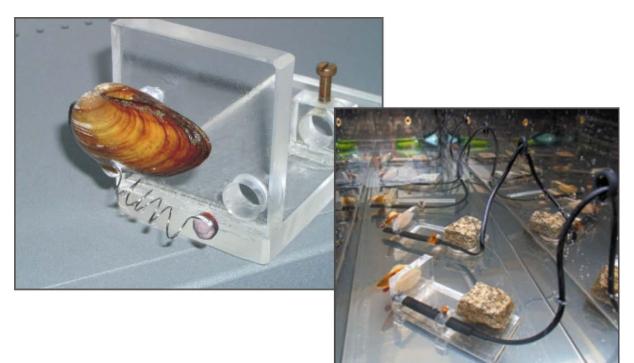
Mussels are not active animals living in the bottom of water reservoirs. Unio tumidus lives in still waters (lakes and ponds) as well as slowly flowing rivers. The majority of specimens can be encountered near lake banks, at the depth of 0,2 - 1,0 m, but they can be also encountered at greater depths. Mussels filter huge amounts of water, whose



constant flow is caused by the work of their ciliated epithelium of the mantle cavity, gills and labial palps. The feeding is passive. Water flows through the incurrent siphon into the branchial chamber. The water carries nutrient particles and oxygen. It rinses the gills and gives oxygen. The ciliated epithelium causes the water to move and the food particles to stay on the surface of gills. Then the food moves towards the mouth, surrounded by the so called labial palps. Mussels' food are particles of detritus (dead organic matter), small plankton organisms and bacteria. The constant flow of water guarantees the cleaning of the branchial chamber of the animal's excrements. The water with excrements flows out of the mussel through the excurrent siphon. An element that might facilitate the flow of water through the mussel's body is the movement of its valves. The speed of mussel shells movement and the quantity of these movements shows the activity of the mussel.

6.2 Functioning of mussels in the SYMBIO system.

In the SYMBIO Biomonitoring System 8 specimens of mussels are used in the system for the period of three months. This period is optimal for their functioning in the system, without the necessity to feed them. After three months the mussels are replaced with other specimen, and the mussels working in the system to date go back to their natural habitat.



7. SYMBIO system operation

SYMBIO Biomonitoring System is maintenance free. System operation is reduced to using a computer program, that is to observe the current mussel openness level (bar graphs), mussels activity over a chosen period of time (line graphs) and viewing the archived data (reports). An exception are situations in which there was an alarm signalized or situations which might cause system disruptions. All the actions necessary to guarantee the proper working of the system and its reliability, such as catching biological material, its transportation, and acclimatization as well as servicing the system (mussels installation, cleaning the tank, service works) are performed by PROTE company.

7.1 Catching biological material

Mussels in the SYMBIO system come from water reservoirs which are subjected to anthropogenic pressure only in minimal extent, that is reservoirs which do not have direct inflows of pollution from a water catchment used for farming purposes. A proper selection of the water reservoir is very important as the indicating organisms have to be highly sensitive to any environmental changes. In the place where the mussels are caught a selection of organisms is carried out, with regard to the mussel age, size and condition. Specimens of a similar size are selected (bigger specimens are preferred). They should not be morphologically different from the rest of population. Mussels transportation is carried out in lake water, in specially prepared and thermally insulated container.



7.2 Acclimatization

It is necessary to carry out acclimatization of mussels in order to minimize their stress resulting from a change in environmental conditions. After the mussels are caught and transported to the laboratory, they are placed in aquariums. At the beginning of acclimatization the temperature of water is set so that it is similar to the temperature of water in the lake, in which the mussels were caught. Before placing the mussels in the system at the Client's the water temperature is gradually adjusted to the temperature in the water intake, where the mussels are to be installed. The water in aquariums is oxygenated so that bioindicators have optimal oxygen conditions. Acclimatization lasts minimum 2 weeks.



7.3 Placing the mussels in the system and their replacement

Mussels replacement with a new set is carried out every three months and it embraces all necessary service works (cleaning of the tank, calibration, carrying out settings of the program), which are performed by PROTE specialists. Each time after mussel installation in the system a hand calibration

is carried out. Next within a few days the system verifies the hand settings and carries out autocalibration.

8. Certificates, honorable mentions and references



SYMBIO Biomonitoring System

Basic data

WE KINDLY ASK YOU TO ANSWER THE FOLLOWING QUESTIONS

ON THE BASIS OF YOUR ANSWERS WE WILL PREPARE AND SEND YOU A NON-BINDING OFFER FOR BIOMONITORING SYSTEM INSTALLATION FOR YOUR COMPANY

| ١. | Data: | | | | | | | |
|-----|---------|-----------------------------------------------------------------------------|--|--|--|--|--|--|
| | 1. | Name and surname | | | | | | |
| | 2. | Post | | | | | | |
| | 3. | Name of the company | | | | | | |
| | | | | | | | | |
| | 4. | Address | | | | | | |
| | 5 | Telephone: | | | | | | |
| | 5. | fixedmobile | | | | | | |
| | 6 | E-mailfax | | | | | | |
| | | Source of information about us | | | | | | |
| | | What branch magazines do you read? | | | | | | |
| | 0. | | | | | | | |
| | | | | | | | | |
| II. | Inf | ormation about the water intake: | | | | | | |
| | 1. | 1. Short characteristics of the water intake (type, water intake way): | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | 2. | Parameters of raw (untreated) water (scope of fluctuations during a year): | | | | | | |
| | | Turbiditytemperature | | | | | | |
| | | Parameters characteristic for a particular water intake: | | | | | | |
| | | | | | | | | |
| ш. | Ge | neral questions: | | | | | | |
| | 1. | Do you use an early warning system about the contamination of the water fed | | | | | | |
| | into th | e water supply system at your water treatment station? | | | | | | |
| | 🗆 yes | 🗆 no, because | | | | | | |
| | | | | | | | | |
| 2. | If you | use a biomonitoring system, what system is it? | | | | | | |
| | | | | | | | | |

since when.....\..... and what are the effects? (Please give a mark 1-5, where 5 – very good)

3. Would you like to take part in a non-binding multimedia presentation, in order to get acquainted with an effective, cheap and fully automatized biomonitoring system?

□ yes □ no, because.....

.....

4. In case of confirming the effectiveness and reasonable cost of our biomonitoring system, would you be interested in using the system at your company?
□ yes □ no, because......

.....

ATTENTION!!!

We kindly ask you to send us this questionnaire by mail, fax or e-mail. We would like to thank all Companies who have sent us filled in forms. We invite you to cooperation aiming to help you improve the safety of your water intakes.