Biomonitoring in the control system of biological wastewater treatment in pulp and paper industry

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Abstract. Modern development of industrial enterprises, economic stability of their work are directly related to the solution of environmental issues arising in the processing of raw materials. The Arctic region is the center of concentration of pulp and paper production, characterized by a large output of various types of commercial products. Currently, international and domestic requirements for the quality of waste, waste water and steam and gas emissions are increasing. In this regard, the control system of biological wastewater treatment of pulp and paper production pays great attention to biomonitoring. One of the most effective methods of wastewater treatment is biological treatment using biocenosis of microorganisms – activated sludge. Using a system of integrated biomonitoring in assessing the state and structural features of activated sludge, whose representatives have the ability to respond to the composition and properties of treated wastewater, regulated by the operation of facilities, it is possible to effectively manage the wastewater treatment system of pulp and paper production.

1. Introduction

Pulp and paper industry is one of the most water-intensive sectors of the national economy. The amount and degree of pollution of industrial wastewater depends on the type of products produced, the capacity of the enterprise, the perfection of the technological process and the production scheme. Wastewater of pulp and paper enterprises contains huge amounts of suspended and dissolved substances of both organic and inorganic origin.

One of the most effective methods of wastewater treatment is biological treatment (BT) using biocenosis of microorganisms – active sludge (AS). This method consists in the ability of different types of AS microorganisms, under certain conditions, to absorb and assimilate pollutants, using them as food and obtaining energy to ensure their functioning.

Effective and efficient control of BT work is only possible with appropriate control of waste water and the BT process. Industrial control at BT facilities includes:

– control of technological parameters of BT process;
– chemical control: inflow control; outflow control; step-to-step BT control of wastewater;
biomonitoring system: hydrobiological AS analysis, calculation of biodiversity index, microbiological AS analysis, enzymatic AS analysis, determination of outflow toxicity, bacteriological control [1].

The tasks of the technological control at the treatment facilities are: evaluation of the effectiveness of the wastewater treatment process, identification of the causes of process abnormality, timely registration of quality treatment deviations from the project or regulatory indicators and recommendations development for complaint’s elimination.

The conclusions obtained on the basis of hydrobiological analysis, the main one in the biomonitoring system, may not coincide with the data of hydrochemical control, as hydrobiological analysis reveals violations more quickly. At present, in the system of biomonitoring of natural and artificial hydroecosystems, including the AI system of biological treatment, there are modern informative ways to assess the functional state of aquatic biocenoses, significantly dependent on the composition of wastewater.

2. Hydrobiological AS analysis
Like any biological system, AS microorganisms is adapted to environmental conditions and to inflow composition.

The treatment effectiveness essentially depends on the structure and biological properties of active sludge. The structural features of floc formers include compactness, density, size and flocculating properties of the bacterial community. Also protozoa may be present in the active sludge. The natural selection of certain microorganisms forming biocenosis provided regime treatment is occurred in non-sterile aerotank conditions using multicomponent organic complex of industrial wastewater (polysubstrate).

The AS state is controlled by hydrobiological analysis. In part, this method can be called microbiological, as it examines the bacterial mass of AS in the form of floc formers, as well as analyzes the protozoa. Hydrobiological analysis consists the microscopic evaluation of conditions and structural features of the active sludge biocenosis. The biocenosis organisms have the ability respond (qualitative change and quantitative distribution of individual groups) to the composition and properties of treated wastewater, as well as to life-sustaining environment, regulated by the industrial regime.

Hydrobiological analysis consists of several stages: determination of the AS concentration, sludge index calculation; microbiological studies of AS including visual sludge examination (sedimentation and microscopy) with a description of the floc formers and microorganisms-bioindicators. The main conclusion includes active sludge activity and its ability to treat pollutants.

3. Microscopy. Microorganisms-bioindicators
Active sludge is a complex environmental system. The active sludge biocenosis includes representatives of six groups of the microflora: bacteria, fungi, diatoms, green, blue-green, euglena microalgae, and nine taxonomic groups of microfauna: flagellates, aschelminthes and coelomate, Gastrotricha, tardigrades (Figure 1 A), ciliates (Figure 1 B), rotifers (Figure 1 C), arachnids, sarcodic (Figure 1 D).

For the correct characterization of the active sludge biocenosis it is necessary to characterize both the state of bacterial populations – the main pollution destructors, and protozoa. The feeding method the active sludge organisms determines their structural position in the biocenosis and the character of mutual relations.

The predominance of individual organism’s group of with a certain nutrition type indicates the structural changes in the biocenosis and allow to suggest possible changes in the environmental conditions of the sludge.

3.1. Biodiversity evaluation
The active sludge organisms are combined into indicator groups with following calculation thereof for correlation the hydrobiological results with the biological treatment process and technological regime. The bioindicators parameters are determined for each biological treatment facility. The universal parameters of the bioindicators for all biological treatment process is cannot be constructed. Some text.

![Figure 1](image-url)

**Figure 1.** Microorganisms-bioindicators of the active sludge: A – Tardigrada (with eggs), B – Epistylis, C – Rotifera, D – Arcella. Scale bars 100 µm.

The species (taxonomic) diversity of the community is an indicator of its ecological condition. The enhanced diversity with 5–6 or more species (taxons) are formed under optimum conditions. This biocenosis provides polydominant properties. In the communities living in extreme conditions, as a rule, biodiversity is reduced. Such biocenosis has monodominant properties with high number organisms belonging to 1–2 species. The BT system of waste water of pulp and paper mill at high load can be an example of such biocenosis. Most often, The Shannon index, Berger-Parker index or Zhmur index are used for BT system monitoring.

4. **Enzymatic analysis. Determination of dehydrogenase activity**

The quality of the treated wastewater may be suitable (based on the chemical analysis) under unfavourable regime, however hydrobiological analysis immediately detects floc formers destruction, changing of biocenosis and physiological conditions of the organisms. The delay of hydrobiological parameters is due to the fact that the internal enzymes provide treatment under floc destruction. Nevertheless the biomonitoring of the BT process depends on the efficiency of analysis and results.

Express-methods application is very useful for improvement the efficiency of biological treatment monitoring. One of these methods is determination the enzymatic activity of active sludge microorganisms. NArFU scientists developed a technique for determining dehydrogenase activity,
based on the measurement of the rate of methylene blue in the redox reaction catalyzed by enzymes. The method allows to instantly determine the oxygen regime of the BT process based on the aerobic coefficient and the value of the reducing capacity.

In on-line mode, this method almost completely eliminates the influence of the subjective facilitation on the analytical results.

5. Determination of the AS bacterial population

The high temperature, especially in summer, presence of recalcitrant substrate, peak loads (wastewater volley), toxicants are characteristic for most pulp and paper mill wastewater. As a rule, pulp and paper mills are township-forming enterprise, consequently their treatment facilities receive household waste water, storm water, waste water of various enterprises, which may contain the amount of pollutants commensurable with the mill’s volume. In summer there are no micro-organisms-bioindicators. It is extremely difficult to control the operation of BT facilities in these situations.

The microbiological analysis becomes the method for identification of the bacterial population of active sludge. Bacteria have advantages in the process of substrate assimilation in comparison with protozoa and multicellular organisms: the smallest size of bacteria cells, larger contact surface with nutrients, shorter generation time. Therefore, bacteria react faster to changes in the environment in which they live. The main physiological groups of bacteria of the activated sludge during biological treatment system of the pulp and paper mill are cellulose-decomposing, denitrifying, nitrifying, nitrogen-fixing, sulfur-oxidizing, desulphying bacteria such as Actinobacteria, Pseudomonadales, Archaea, Chloroflexi, Firmicutes, Cyanobacteria and other species.

During the stable BT process with floc formers of active sludge under average load are compact. Often the filamentous are matrix of the active sludge in pulp and paper industry (Figure 2 A). The active sludge bulking appears during changing of wastewater composition, regimes of BT process or intoxicant presence (Figure 2 B). The bulking should be promptly eliminated.

![Figure 2. Floc formers of active sludge. A – floc formers of active sludge under average load, B – filamentos and zoogleal bulking of active sludge, C – biofilm of MBBR-reactor. Scale bars 100 µm.](image)

NArFU Biotechnology group developed a method of quantitative determination of filamentous and zoogleal forms, which allows to visualize the changes in the bacterial population of active sludge. The famous methods for determination of cell number and morphology thereof are direct counting or plate count method according to Koch. But these methods have disadvantageous features. The definition of direct counting gives the total content of bacteria in the active sludge, regardless of their physiological group. This method takes into account both living and dead cells. When calculating the number of bacteria by the plate count, only living cells are determined, but not all groups of bacteria grow on the media.
Modern and most accurate method of identification of bacteria’s species is metagenomic analysis. Metagenomic analysis is a method to determine the qualitative and quantitative composition of the microbial community. Each microorganism has in its DNA a encoded unique genetic sequence called 16s rRNA. By 16s rRNA it is possible to determine the species of the microorganism. During the metagenomic analysis, the total DNA of all microorganisms is extracted from the sample, and the reading of the 16s rRNA gene fragment is performed. After data processing readings are compared with already known ones from international databases, and the number of representatives of each taxonomic unit is calculated [2].

6. Toxicity Determination
The biotesting of treated wastewater (outflow) is realized during industrial analytical control of BT process, in addition to hydrochemical and hydrobiological analysis. Also, there may be biotesting of wastewater (inflow) for identification of toxic effects on active sludge and providing high efficiency of the enzymatic oxidation of pollutants.

Biotesting is using of biological objects (test objects) in controlled conditions for identification and evaluation of the factors (including toxic) of the environment on the organism, its individual function or system of organisms [1].

During ecotoxicological control in Russia, it is proposed at least two standard methods of biotesting with test organisms from different systematic groups. Methods of biotesting chosen in such a way that hydrobionts should be from different trophic levels, but with different sensitivity to various pollutants. The toxicity of pulp and paper waste water is determined by the vital activity of daphnia Daphnia magna or ceriodaphnia Affinis ceriodaphnia, but the most sensitive method is to determine the toxicity of water by the chemotoxic reaction with infusoria Paramecium caudatum.

7. Bacteriological monitoring
The bacteriological parameters of domestic wastewater are monitored at the mechanical treatment location and at the outflow. The following parameters are defined: total coliforms, coliphages, thermotolerant coliform bacteria, pathogenic bacteria, viable helminth eggs. Systematic bacteriological control of outflow wastewater guarantees the epidemiological safety of the population.

Effective and efficient control of BT process is possible with appropriate monitoring of waste water. The main role in a BT monitoring system is given to biomonitoring. Evaluation of the composition and structural features of the biocenosis active sludge, representatives of which have the ability to respond to the composition and properties of treated wastewater, as well as the life support conditions regulated by the operation of facilities, it is possible on the basis of the results of biomonitoring as a complex to effectively manage the wastewater treatment system.

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