

KRZYSZTOF BARBUSIŃSKI\*

## ADAPTATION OF ACTIVATED SLUDGE TO LABORATORY RESEARCH CONDITIONS

Activated sludge taken from the municipal wastewater treatment plants for testing purposes is very much different in its biochemical and physical properties from the one from laboratory. This is not only due to the different chemical composition of wastewaters, but also due to the different scale of technological equipment making it impossible to reproduce such process parameters as degree of oxygenation, hydrodynamic conditions in the aeration tank, recycle ratio and sludge age. Transferring the biocenosis of the activated sludge into a different environment is connected with more or less serious disturbance in the internal balance of the biocenosis and with the necessity of adapting the metabolism and/or composition of the species to a new environment. The preliminary adaptation process of the activated sludge has a direct influence on the results of experiment conducted and on the proper interpretation of the relationships identified. Therefore, adoption of adequate criteria for the state of adaptation, which could automatically impose limitations on the duration of the primary adaptation period, is the major problem. The criteria should include basic parameters characterising both biochemical and physical properties of the sludge.

In this paper, the problems of adaptation of activated sludge microorganisms to the laboratory conditions are discussed. An attempt to standardize the principles of the preliminary adaptation of the activated sludge in order to provide optimal conditions for the experiment planned is also made.

### 1. INTRODUCTION

In the processes of biological waste treatment, most of scientific experiments and researches are carried out in the laboratory. Activated sludge used in these experiments is taken most often from the aeration tanks or secondary settling tanks of the municipal wastewater treatment plants, and then adapted in the laboratory to a particular process conditions. Transferring the biocenosis of the activated sludge into a different environment is connected with more or less serious disturbance of the internal balance and with the necessity of adapting the metabolism and/or composi-

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\* Institute of Water, Sewage and Waste Technology and Engineering, Silesian Technical University, 44-100 Gliwice, Kuczewskiego 2, Poland.

tion of the species in the biocenosis to the new environment. The environmental changes may also influence the process of flocculation as well as the sedimentation characteristic of the sludge [10], [13]. The process of adaptation of the activated sludge microorganisms to the new conditions may be carried out in different ways, depending on the individual approach of the researcher to the problem of adaptation, and on the experiment design and methodology as well as the number and kind of the parameters controlled [9], [16], [21].

Additional difficulties are due to the complexity of the adaptation process and the lack of the precise definition of the notion of adaptation. Generally, according to the encyclopedic definition, adaptation is the process of producing the physiological and structural changes allowing microorganisms to take greater advantage of the outer environment conditions or providing greater independence from its harmful effects. This definition, however, does not provide a definite approach to the solution of the adaptation problem which, despite the researchers' agreement in some of its aspects, is considered in unequally detailed manner.

In this paper, the problems of adaptation of activated sludge microorganisms to the laboratory research conditions are discussed. An attempt to standardize the principles of performing the preliminary adaptation of the activated sludge in order to provide optimal conditions for the experiment planned is also made.

## 2. ADAPTATION OF ACTIVATED SLUDGE TO LABORATORY CONDITIONS

Activated sludge taken from the municipal wastewater treatment plants for testing purposes is very much different in its biochemical and physical properties from the one existing in the laboratory. This is not only due to the different chemical composition of wastewaters, but also to the different scale of technological equipment making it impossible to restore such process parameters as degree of oxygenation, hydrodynamic conditions in the aeration tank, recycle ratio and sludge age. The research into the problem of rescaling the technological devices in biological processes has been carried out for some time.

Biological properties, in particular the values of different constants in the equations of fermentation kinetics, depend on the scale of the equipment [2]. Therefore, maintaining identical process conditions in both industrial and laboratory scales should be the essential feature of scale change process. These conditions being identical does not necessarily mean the similarity of the microorganisms activity, as the metabolism of microorganisms is the result of a number of complex factors impossible to be recognized by means of simple analyses of the process conditions. Moreover, the direct relationships between the microorganism activity and the process conditions represented by the scale change criteria are still not fully recognized. The above facts prove that the microorganism behaviour in different

scale processes is not simple and does not fit the usual concept of the system similarity in hydrodynamic sense [2].

The results presented reveal that the transfer of a particular microorganism biocenosis from a definite technological system to laboratory conditions does not ensure full similarity of the activity and individual functions of the microorganisms, even in the case when no nutrient change occurred, basis process parameters are maintained and the geometric relationships in the model systems are preserved.

Lack of definite solutions in several basic problems and their high complexity cause the omission of a number of principles of transfer theory in the biological wastewater treatment practice, both at the design stage and during laboratory research, and the adaptation of activated sludge biocenosis to laboratory conditions is treated rather schematically. Most often it is assumed that after a specific time elapsed, when the microorganism culture has grown in the new environment under relatively stable conditions close to those of the mother process, the activated sludge adaptation is sufficient. Another adaptation criterion often accepted is the time required for the activated sludge to reach the assumed level of substrate removal. Such a process is defined as activated sludge maturing and is often identified with the adaptation process.

Many authors of scientific publications do not give any information on the stage preceding the main experiment and in the case when such an information is given, it is often much too general and imprecise. This proves that too little attention is paid to the problem of proper adaptation of activated sludge microorganisms to laboratory conditions.

## 2.1. PRELIMINARY ADAPTATION PROCESS

The preliminary adaptation of the activated sludge has a direct influence both on the results of experiment performed and the proper interpretation of the relationships established. The investigations of the activated sludge process under conditions of dynamic substrate load changes have shown that when no preliminary adaptation has been performed or it was too short, the time the activated sludge reacted to the changes in environmental conditions was delayed and the relationships defined were much different from those characteristic of the processes with correct preliminary adaptation [4], [5].

Publications providing information on the preliminary adaptation of microorganisms to the test conditions may be divided into several classes. In the papers of the first class, only the time necessary for adaptation is recommended.

SUSCHKA [20] recommends 20–30 day preliminary period of activated sludge microorganism adaptation before starting the proper laboratory experiments. TSUNO et al. [22] have proposed a kinetic model simulating the substrate utilization, the metabolism and the oxygen consumption rate in the activated sludge process. The model allowed us to predict the sludge reaction to qualitative overloads in

a completely mixed system. The activated sludge from a municipal wastewater treatment plant was being subjected to adaptation during at least one month. BARAHONA and ECKENFELDER [3], who investigated the sedimentation properties of the activated sludge over the substrate load range of 0.1 to 0.6 g BOD<sub>5</sub>/g·d, ensured separate 5-week adaptation for each load (in separate laboratory units). JONES et al. [11] have tested the influence of different substrate additives on the ability to remove phosphorus from municipal wastewaters by the activated sludge under laboratory conditions. In order to ensure microorganism adaptation and stabilization of the process parameters, the activated sludge has been matured for two weeks.

Another class include the papers the authors of which adopt very strict criteria of the adaptation stage or they just define the way in which the process should be performed. This information does not always provide the real duration of this stage.

KRISHNAN and GAUDY [12] investigated the reaction of the activated sludge to rapid qualitative changes of load in two parallel continuous systems with and without biomass recycling. Each system was running at least three days under steady-state conditions, which were referred to biomass and substrate concentration before the experiments.

Similar research was conducted by SHERRARD and LAWRENCE [19], who used a laboratory completely mixed reactor with integrated central settling tank, from which the activated sludge was recycled automatically due to forced wastewater cycle. Different values of hydraulic retention times and sludge age were applied. Before each experiment, microorganisms were grown during circa 1 week at the given values of retention time and sludge age. This time was sufficient for the suspended microorganisms to reach the balance.

After the stabilization of the biomass concentration had been reached, the system was considered to be at the steady-state. Thereafter it was subjected to rapid step substrate loads without additional period of stable operation under these conditions. CHEN et al. [6], similarly as other researchers, took the activated sludge samples from the municipal wastewater treatment plant. The adaptation period applied was sufficient for the microorganisms to reach the best activity in substrate biodegradation. MIKSCH [14] carried out the experiments aimed at proving, whether the effect of homeostasis occurs in heterogeneous microbial populations of the activated sludge type. The adaptation in this case was performed in a continuous way, with steadily increasing substrate load of microorganisms, until the assumed and suitable value was reached.

The third class includes the publications in which the most extensive and relatively clearly given criteria of the adaptation process are given.

ADAMS and ECKENFELDER [1] were investigating the behaviour of the activated sludge under conditions of variable substrate loads. The experiments were conducted using synthetic, municipal and industrial wastewaters, with two- and threefold load increase and in four-, eight- and twelve-hour transient period. The adaptation cycle

of the microorganisms for each kind of wastewaters lasted until the effects of pollution, i.e. COD, oxygen uptake rate and biomass growth rate, stabilized at a constant level for at least one week.

SELNA and SCHROEDER [18] investigated the reaction of the activated sludges of different ages to short, five-hour substrate overloads. The preliminary period of the system remaining under steady-state conditions, necessary for appropriate adaptation of the activated sludge biocenosis, was defined as a one-week period, during which the suspended solids of the sludge, effluent suspended solids and COD values of the treated waste were relatively constant. For example, maximum variability of the suspended solid value under these conditions was 8%.

DĘBKOWSKA and BODZEK [8], investigating microorganism adaptation to conventional process conditions of the activated sludge at a wide range of analytical control (oxygen uptake rate, dehydrogenase activity, catalase content, COD, sludge volume index, settleability), found that the period necessary for stabilization of these values is two weeks.

## 2.2. CRITERIA FOR STATE OF ADAPTATION

Activated sludge can be regarded as adapted to experiments when it does not show any changeability of its biochemical and physical features. To carry out optimum preliminary adaptation of the activated sludge it is necessary to ensure steady conditions of the process, the conditions being as close to the real experimental conditions as it is possible. It is especially important to keep the values of the substrate load of the activated sludge, its reaction and the content of dissolved oxygen in the aeration tank at a steady level.

The authors' research and literature data confirm the statement that under such conditions the evaluation of the state of adaptation should be carried out on the basis of the control of the effects of substrate removal, biomass growth, oxygen uptake rate and/or dehydrogenase activity (TTC test) and sludge volume index. To supplement these, microscope examinations should be done to estimate changes taking place in the biocenosis of the activated sludge.

The authors' experiments [5] proved that dehydrogenase activity is that parameter which reaches steady values as the first one informing of adaptation of the enzymatic apparatus of sludge. Stabilization of the sludge volume index shows that physical changes have finished. Depending on the fact whether transferring a given biocenosis of the activated sludge to a new environment causes more or less stressful conditions, the deferment of a reaction of physical properties in relation to biochemical properties can be changeable, thus limiting the time of adaptation.

Generally, the period of preliminary adaptation can be considered to be finished when the range of changes of all analysed quantities within a few days does not exceed 10–15%. The biocenosis of the activated sludge should be characterized by the stability of its composition as well.

### 3. DISCUSSION

The literature review presented reveals differences in the approach to the problem of activated sludge adaptation to the laboratory conditions. Individual authors define different criteria and different times of the preliminary adaptation period. The information given is often much too general and practically in no case the grounds for the criteria defined are given. In the case of information concerning only the time of the preliminary adaptation, it is difficult to say whether any analytical control has been provided, or whether the information is so limited due to little attention devoted to this part of the experiment. It must be stressed, however, that the preliminary adaptation period is an integral part of the whole investigation process and thus should be treated with adequate attention.

In order to estimate the degree of activated sludge adaptation, it should be taken into account that the environment changes are caused by the changes in both biochemical and physical properties of the activated sludge. Therefore, the assessment of the degree of adaptation of the activated sludge based on one parameter value only is not proper. On the other hand, control of a large number of parameters is often impossible due to the technical difficulties and even is not reasonable. For example, according to LAUBENBERGER [13], the following parameters of the activated sludge should be considered: biochemical activity, substrate removal rate, sludge growth, its settleability and dewatering ability. Also the assessment of the structure and active surface of flocs may be helpful in this case [13]. The adaptation process can be more precisely characterized by the measurements of the synthesis of high-energy compounds (ATP), nucleic acids, and – in some cases – adaptive enzyme level. These could easily lead to a situation when the primary adaptation becomes a research process itself.

Therefore, adopting the adequate criteria for the state of adaptation is the major problem. These criteria could automatically impose limitations on the time of the primary adaptation. They should include basic parameters characterizing both biochemical and physical properties of the sludge. When the research is aimed at determination of unique properties, or carried out within a wide analytical range, this range should obviously be extended.

The basic criteria proposed include the control of the substrate removal effects (as the main aim of biological waste treatment), oxygen uptake rate and/or dehydrogenase activity, reflecting general physiological state of the microorganisms, as well as the volume index of the activated sludge. General physiological state of microorganisms may only be assessed basing on the processes of a substantial significance for the whole microorganism cell. For this reason biochemical oxidation proceeding with active enzymes of the respiratory chain, where basic metabolic processes are integrated, is of a particular importance. Thus, with time sequence taken into account, it may be assumed that during conversions causing the changes in the intensity of vital processes, the variation in the activity of respiratory enzymes is the earliest signal of the change [15]. The sludge volume index is a parameter not only characterizing sedimentation

properties of the sludge, but it is also interrelated directly with other physical properties such as floc sizes, sludge density and structure, its dewatering ability, etc. [7], [17]. It is also recommended to carry out periodic microscopic examinations in order to determine possible changes in the biocenosis of the activated sludge as well as stabilization of its composition.

Besides the above basic criteria, it is also necessary to measure these quantities which are the main aim of the experiment. For example, if one investigates the possibility of intensifying the phosphorus removal from wastewaters, the phosphorus content and degree of its removal must be determined as early as at the preliminary adaptation stage. It is desirable that the removal is stable, or in proportion to the changes in influent concentration.

As the adaptation process should proceed under stable conditions, the parameters limiting directly the value of the substrate load of the sludge (i.e., the concentration of the substrate feeding the system, its retention time and biomass concentration of the activated sludge) should also be controlled and their stability should be maintained. Such parameters of the system as its reaction and dissolved oxygen content should also be routinely tested.

It is obvious that keeping the values of technological parameters constant in the course of biological experiment is fairly difficult and most often the operating conditions of the system correspond to those of the steady state only in theory. For this reason it is necessary every time to set up allowable limits of variability of individual parameters. The process is considered to be in steady state and the system to be adapted when they remain within these limits. It should be noted, however, that the variability may also be due to analytical mistakes. The allowable range of variability should not exceed 10–15% in any case. As a general rule, the more precise the values of individual parameters determined in the course of experiment and the more reliable their interdependences are demanded, the more narrow an allowable variability interval in the preliminary period should be. The basic quantity characterizing the conditions of the activated sludge process is its substrate load. Thus, in the case of difficulties in maintaining the process stability in the preliminary period, it is possible to establish a criterion which allows assessment of the degree of adaptation of the system, i.e. a ratio of the changes of the parameters controlled to the load changes.

The activated sludge is sufficiently adapted when the stability of the parameters determining the degree of adaptation is maintained for several days. This period is also necessary for determining the reference values for the individual quantities being a subject of further research.

#### 4. CONCLUSIONS

Each report on research result should include the description of the preliminary adaptation process. In such a report there should be established the criteria applied and the allowable variability values accepted. Some general remark should also be

given on the way in which this stage of the process has been carried out. The precision of such a description is of a particular importance in the case of basic research which allows us to verify the experiment results and to obtain an adequate repeatability, as the theories and relationships may be considered proved if the results were confirmed by other experimenters in their laboratories under the same conditions.

The discussion presented is an attempt to standardize the rules governing the preliminary adaptation of the activated sludge under laboratory conditions. The author's intention was to indicate the importance of the problem and to make an approach to its methodical solution.

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## ADAPTACJA OSADU CZYNNEGO DO WARUNKÓW LABORATORYJNYCH

Osad czynny z miejskich oczyszczalni ścieków często znacznie różni się pod względem biochemicznym i fizycznym od osadu pracującego w warunkach laboratoryjnych. Wpływa na to nie tylko inny skład chemiczny ścieków, ale także różnica skali urządzeń technologicznych, która powoduje, że w warunkach laboratoryjnych nie jesteśmy w stanie odtworzyć między innymi takich parametrów procesu, jak: stopień natlenienia, warunki hydrodynamiczne w komorze napowietrzania, stopień recyrkulacji i wiek osadu. Przeniesienie biocenozy osadu czynnego do innego środowiska powoduje bardziej lub mniej poważne zakłócenia równowagi wewnętrznej biocenozy i konieczność przystosowania jej metabolizmu i/lub składu gatunkowego do nowego środowiska.

Proces wstępnej adaptacji osadu czynnego ma bezpośredni wpływ na wyniki eksperymentów i prawidłową interpretację otrzymanych zależności. Dlatego podstawowym problemem jest przyjęcie odpowiednich kryteriów stanu zaadaptowania, limitujących automatycznie czas trwania wstępnego okresu adaptacji. Kryteria te powinny obejmować podstawowe parametry charakteryzujące zarówno biochemiczne, jak i fizyczne cechy osadu.

Opisano adaptację drobnoustrojów osadu czynnego do laboratoryjnych warunków badań. Podjęto też próbę ujednoczenia zasad prowadzenia wstępnej adaptacji osadu czynnego w celu uzyskania warunków optymalnych w danym zakresie badań.

## АДАПТАЦИЯ АКТИВНОГО ОСАДКА К ЛАБОРАТОРНЫМ УСЛОВИЯМ

Активный осадок из городских очистных станций часто в значительной степени отличается биохимически и физически от осадка, работающего в лабораторных условиях. На это влияет не только другой химический состав сточных вод, но также отличие масштаба установок, которое приводит к тому, что мы не в состоянии воспроизвести между другими таких параметров процесса, как: степень окисления, гидродинамические условия в аэротанке, степень рециркуляции и возраст осадка. Перенесение биоценоза активного осадка в другую среду вызывает более или менее серьезные нарушения внешнего равновесия биоценоза и необходимость приспособления его метаболизма и/или состава пород к новой среде.

Процесс предварительной адаптации активного осадка имеет непосредственное влияние на результаты экспериментов и правильную интерпретацию полученных зависимостей. Поэтому основной проблемой является принятие соответствующих критериев состояния адаптации, лимитирующих автоматически время продолжения предварительного срока адаптации. Эти критерии должны охватывать основные параметры, характеризующие как биохимические, так и физические черты осадка.

Описана адаптация микроорганизмов активного осадка к лабораторным условиям исследований. Предпринят опыт унификации принципов ведения предварительной адаптации активного осадка для получения оптимальных условий в данных пределах исследований.