

THE CONVERGENCE OF BIOTECHNOLOGY AND NANOTECHNOLOGY AS AN ACCELERATOR OF THE DEVELOPMENT OF BIOFILM TECHNOLOGIES

L. Nikolov, V. Mamatarikova, S. Slavchev, S. Stoychev

Abstract. An attempt is made to elucidate some of the existing potentialities offering the convergence of the biotechnology and nanotechnology in biofilm science and technology. This is achieved by submitting information about the main features of the biofilm structures. The current terminology for biotechnology and nanotechnology is also specified and some new notions have been introduced. Using the terminology and presenting in dynamics the formation and functioning of biofilms, the main reason for their qualification as complex systems in terms of the system approach is presented. Special attention is paid as well to the self-organization of biofilms and to the possible intervention into it. On this basis, some of the main points of the convergence of biotechnology and nanotechnology are revealed and the future developments are discussed. The notions of “nanobiotechnology” and “bionanotechnology” are discussed as appropriate expressions reflecting the convergence of these two modern fields of R&D activity in biofilm technologies.

Keywords: biotechnology, nanotechnology, biofilm, nanobiotechnology, bionanotechnology, self-organization

INTRODUCTION

Before formulation of biofilms as convenient subjects of research and development activity, where the biotechnology can converge with nanotechnology, it is necessary to outline their main features in the light of the new conceptions about these modern fields for investigations and implementations. This also can help avoiding ambiguity, because the terminologies in these fields are in continuous modifications or permanent corrections, due to dynamic character of R&D activities in these fields.

As to *biofilms*, they are well-known in science and technology more than century. Because of that, it is quite reasonable to think that the main notions are already established. Although as the last investigations show, the terminology of biofilms is not protected from modifications even of certain changes as well. It is entirely accepted that biofilms are living systems in the forms of layers, consisted of spontaneously fixed cells of microorganisms, plants or animals on carriers (mainly on solid substances); produced by themselves compounds (EPS - exopolysaccharides, DNA, precipitated salts, etc); inclusions of nano- and micro-particles and circulating free cells in their structure [1,2]. They attract the scientist's interest for many years, not only due to their special structure and unique characteristics, but by the potentialities for practical implementations. As the results of a study on the research interest show just in one of their fields of application – the biofilm reactor design, the number

of investigations grows exponentially during the last two decades [3]. Enormous number of investigations is dedicated to biofilms and regularly very prestigious scientific forums are organized. Unique properties and amazing behavior under the condition of “in vitro” cultivation for the needs of high performance bioreactors design and bioprocess systems with fixed film developments, also have been subjects of our R&D activity for a long period [4,5,6,7,8]. A part of these very important features of biofilms as living systems will be scrutinized here below.

Biofilm impacts in natural systems. Biofilms exist in natural ecosystems playing positive role in acceleration of natural cycles of biogenic elements - C, N₂, P, S, O₂. In the high organized living systems – plants, animals and humans their role is controversial. From one side it can be qualified as negative by causing dental plaques or disease's prolongations and from the other – it is positive in acceleration of digestion processes in high organisms [9].

Biofilms in artificial systems: Biofilms role is mainly negative. Here, it is necessary to mention biocorrosion, which provokes a lot of problems in metal protection, decreasing of the working characteristics of equipment and apparatus in: water transport – decrease of the transport equipment speed, heat exchangers - decrease of heat transfer coefficients, artificial organs – plug formations and occlusions etc. This dark side of biofilms has been discussed in our works [10,22]. However there are some domains in the artificial

systems, which can be used to illustrate their positive role. One of the most spectacular of them is the biofilm reactors [11,12] and its implementation in environmental friendly technologies, recently discussed in our paper [8].

From the other side, the biofilms as living system are amongst the most interesting subjects of *biotechnology**. As a interdisciplinary and comparatively new field of R&D activity, the determination of biotechnology has gone through several stages, starting with its formulation given at the Second congress of the European federation of biotechnology in 1981, later criticized as "ill defined" notion [13] and ending with the last definitions accepted originally in 1992 and confirmed in 2008 [14]. According to this document biotechnology is: "Any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use".

It is largely accepted now, that *nanotechnology** started as interdisciplinary field since the lecture of famous scholar Richard Feynman [15], who first attracted the scientist interest to the perspectives of investigations in the nano scale range. Later, Taniguchi [16] gave the name of this field as "nanotechnology" and finally – Kahn [17] has given this field a large popularity by his splendid article. Nanotechnology can be simply accepted as implementations of existing sciences into the nano scale. The high importance of nanotechnology is recognized by the European community government by giving top priority of this field for R&D in the 7th Framework Program - respectively - Activity 4.1. Nanosciences and Nanotechnologies which includes: 4.1.1 Nanosciences and converging sciences; 4.1.2 Nanotechnologies and converging technologies and 4.1.3 Health, Safety and Environmental Impacts [18].

AIM

The aim of this study is to reveal in certain degree the potentialities of unification of bio- and nano- technologies for acceleration of the biofilm technologies using a large amount of

information and data about biofilms, biotechnology and nanotechnology, a part of them already published in our papers.

METODOLOGY

The following methodological approaches for reaching the aim will be used:

- a) current terminology and up-to date definitions for determination of the frontiers of the bio- and nano-technologies and their possible common influence on biofilm technologies;
- b) verbal modeling of the biofilm structure and dynamics of its development for searching points of convergence of the biotechnology with the nanotechnology.

RESULTS AND DISCUSSION

a) Current terminology.

As experience shows, the role of the terminology in solving of methodological problems is very important, especially when it concerns new scientific fields and technologies. Using precise and, in the same time, comprehensive definitions and current terminology, the information obtained is more clear and reliable. In our case, the current terminology could relief understanding of "state of the art" in bio- and nano- technology regarding biofilms. The more reliable information about the main trends in their R&D activity on the matter concerning biofilms will allow easier prognosticating of their possible joint applicability. After above presented definitions, largely accepted in the scientific circles, for the aims of this study, it is necessary to present the newest definitions in these fields or to formulate certain number of new notions when it is necessary.

The biofilm technologies. The controversial role of biofilms in nature and artificial systems induces development of two different groups of methods, on which, respectively, two main types of technologies can be built. In the first group, the biofilm useful properties are implemented, mainly in high performance biofilm reactors, than in the second the main aim is to avoid biofilm formation and development in artificial and natural systems. Thus, two types of biofilm technologies can be

*all definitions and current terminology are directly copied from the INTERNET (<http://en.wikipedia.org>)

formulated in the frames of two branches the industry – *processing*** and *protective***.

*In processing technologies*** the biofilm is used as biogent (biocatalyst) of high level of organization in bioreactors with spontaneous fixed biomass for the needs of: environmental protection in wastewater treatment, tail gas purification [6], potable water conditioning – bionitrification, biometallurgy – metal leaching, bioactive substances production. The very important role of biofilm in the environmental friendly biotechnology is well known. It has been recently discussed in details in series of our works [8,19].

*In protecting technologies*** (also: “preserving”**, “impermissible”** technologies). Here, the knowledge about the biofilms is used to avoid their formation from biocorrosion of metals, from dental plaques formation, for ensuring artificial organs without biofilms, or to destroy biofilms in artificial systems - in potable water supplying systems, water transport equipment protection, nano- membrane without biofilms etc).

Thus, nowadays it is possible to discern two types of biofilm technology in the frames of two main branches of technological classification: processing biofilm technology and impermissible biofilm technology. From the other side, as abovementioned, the biofilms belonging to biotechnology makes biofilm technologies typical its representatives. The up-to date definition allows discerning of the following main branches of modern biotechnology*:

- red biotechnology, i.e. medical sciences and applications
- green biotechnology linked with agriculture and food production
- white biotechnology which includes the industrial realization
- blue biotechnology deals with seas and oceans

Using the above given terminology it is clear that in each of these biotechnological branches it is possible to find biofilms impacts

and respectively to encompass the territories for biofilm technology implementations.

*Nanotechnology new branches**. The very advanced character of nanotechnology, its high level of interdisciplinary and potentialities to attract the interests of specialists from unexpected scientific areas, make it possible to formulate the notion of converging sciences. It includes those scientific fields, in which the nano-scale research can produce advanced scientific results and implementations. One of the first fields, selected as converging is biotechnology. This induced, immediately, almost in parallel, formulation of two new notions – nanobiotechnology* and bionanotechnology*. Their explanation is necessary for determination to what of these two fields to refer our considerations, analysis and conclusions and respectively our expectations.

*Nanobiotechnology** is the branch of nanotechnology with biological and biochemical applications or uses. This is a sciences intersection, which subjects of studies existing elements of nature in order to fabricate new devices. Nanobiotechnology usually refers to the use of nanotechnology to further the goals of biotechnology.

The term of *bionanotechnology** is often used interchangeably with *nanobiotechnology**, though a distinction is sometimes drawn between the two. If the two are discerned, than bionanotechnology might refer to any overlap between biology and nanotechnology, including the use of biomolecules as part or as an inspiration for nanotechnological devices. Bionanotechnology may also refer to the use of biomolecules for applications in nanotechnology. A major example of this is DNA nanotechnology, which uses self-assembling nucleic acid structures to control matter at the nano scale size. In a wider sense, bionanotechnology refers to synthetic technology based on the principles and chemical pathways of living organisms. It encompasses the study, creation, and illumination of the connections between structural molecular biology and nanotechnology, since the development of nanomachinery might be guided by studying the structure and function of the natural nanomachines found in living cells

* these definitions and current terminology are introduced for the first time in the practice of biotechnology

b) Verbal modeling of biofilm structure and dynamics of its development.
Peculiarities of biofilms as large and self-organizing living systems

1. Biofilms as large systems. Structures and links.

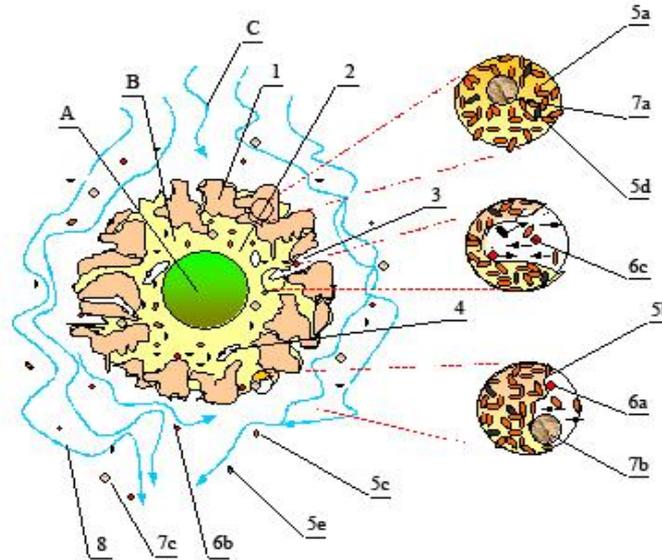


Fig.1. Stylized schemes of biofilm systems - mature biofilm on spherical carrier [20]. A - *carrier*, B - *biofilm*; C - *fluid*; 1 - clusters, 2 - exopolysaccharides, 3 - channels or pores, 4 - closed rooms, 5 - cells, 5a – cells in biofilm, 5b – cells on the biofilm surface, 5c – suspended cells, 5d – dead cells in the biofilm, 5e – dead cells in the liquid flow, 6 – microparticles, 6a – micro particles on the surface of biofilm, 6b – suspended micro particles, 6c – micro particles in pores, 7 - inert macroparticles, 7a - inert macroparticles in biofilm, 7b – inert macroparticles on the surface of biofilm, 7c – suspended inert macroparticles, 8 - stream lines. [20]

Scheme on Fig.1 reflects a comprehensive verbal model of the already formed biofilm structure, where all its principal elements can be seen. This model, based on the current conceptions of biofilms, has been created and modified during a rather long period starting with investigations since 1985 [21] and ending recently [8,10,22,23,24]. The structure shown is of a mature biofilms, as a result of rather long time of its formation, continuous growth and, in the same time, of functioning of its constituents. Some of the most interesting formations as results of these processes are presented on Fig.2 [20], where the influence of the space around the biofilm surface and the conditions, provoked by the liquid flow. Attention is paid to some of elements in certain biofilms like those in the water supply municipal systems and heat exchangers, where piques and streamers can be observed. Their shape and behavior are strongly dependent of the liquid flow.

Fig.3 [20] presents well arranged patterns of the cells states. It can be seen the existence in the layers some inactive cells and cells with various levels of strength of their links with the already settled biofilm structure.

These examples illustrate a very complicate structure as a whole. It is necessary to underline that the phenomena of physical (diffusion, adhesion), physico-chemical (adsorption) and biological (growth) nature run simultaneously in the space of biofilm and in the liquid flow exerting very strong influence one another and giving as a result a very complicated living system [25,26]. As the analysis show, the biofilm must be considered together with the space around its surface, i.e. with the liquid phase as united entity. There, the biofilm formation and functioning on the carrier surface occur in parallel with the processes in the liquid flow, where the living cells are under the conditions of pseudo homogeneous suspended cells

cultivation. Thus, in every biofilm system two linked one another subsystems exist – one of the liquid phase, subordinated to the principles of suspended cells cultivation and the other –

on the carrier surface, i.e. the biofilm, that subsists due to an amalgam of phenomena named as “spontaneous fixation” [10, 22].

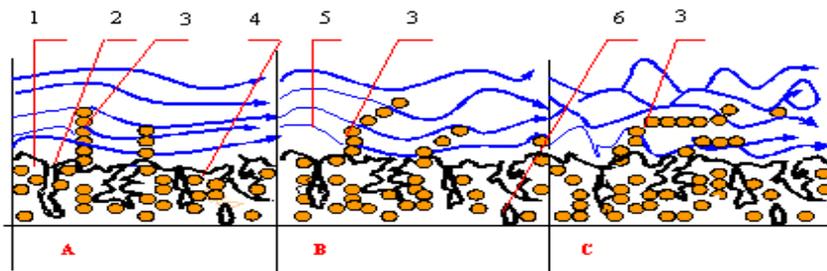


Fig. 2. Formation of biofilm structures on the frontier with liquid phase at different scales of liquid flow linear velocity. [20]. A - low velocities ; B - middle velocities ; C - high velocities.

1 – piques on the biofilm surface; 2 – pores (channels); 3 - streamers; 4 - microorganisms; 5 – liquid flow lines; 6 – closed empty room.

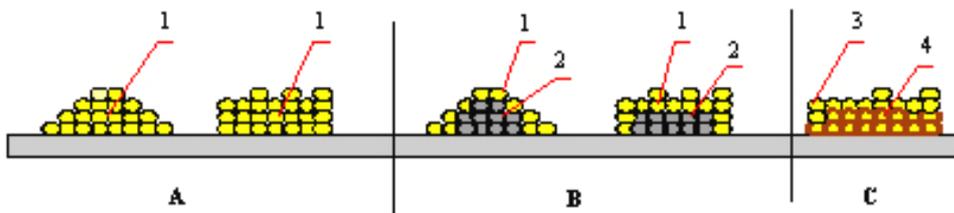


Fig. 3. Various states of fixed cells at the beginning of biofilm formation. [20]. Structures A and B with active (pos. 1) and inactive (pos.2) cells and structure C – weakly linked cells (pos. 3) and strongly bounded microbial cells (pos. 4)

These two systems are strongly linked each other ensuring the living cycle of biofilm existence and making possible realization of the rather complicated multistage mechanism of substrates bioconversion to products, carried out under the conditions of homogeneous-heterogeneous cell cultivations. The scrutinizing of the main stages of this mechanism, which reflects only one side of the bioprocess kinetics, without taking into account the biofilm growth, can be useful as a source of information about the main events in the kinetics of substrates consumption and products formation. Description of this mechanism can be treated from modeling point of view as a partial verbal model of one of the main constituents of the overall kinetic scheme of biofilm functioning. Presented here below, this model does not concern attracting and

inclusion of macro and micro particles (Fig.1, pos. 6 and 7) in biofilm structure, assuming that these phenomena are not typical for all the set of biofilms, i.e. they can not be observed in all biofilm formations. It gives the additional possibility for model simplification.

Thus, keeping in mind all the simplifications, one might say that the partial model developed reflects the main stages of homogeneous-heterogeneous mechanism of bioconversion. They can be marked and very shortly commented as follows: 1) *Substrates mass- and heat- transport from the main body of liquid flow to the biofilm surface.* Usually the transport phenomena in this part of the system space are realized under the conditions of convective external diffusion; 2) *Substrate quasi molecular diffusion in the depth the biofilm layers.* This type of transport phenomena in the gel-like

media, to which the biofilm textures usually refer, is characterized with the coefficients of effective diffusion and heat transfer through porous media; 3) *Substrate penetration through the cell membrane and its conversion in the biofilm cells to products*. This stage can be discerned to many simple steps, but for the aims of simplification of modeling, they are incorporated in one whole with a general velocity of substrate conversion. Along with the conversion of substrates to products, a certain amount of heat is produced, due to the overall calorific effects of biochemical reactions; 4) *Product diffusion from the cell back to biofilm surface*. The transport phenomena are of the same type like in the stage 2; 5) *Product transfer from the biofilm surface back to the liquid flow main body*. The transport phenomena repeat those of stage 1 in the reverse order.

The simplified partial verbal model of the substrate conversion into the product treats only the biofilm contribution to the overall conversion in the common homogeneous-heterogeneous living system, ignoring the substrates conversion in the liquid phase. This can be legally admitted, because when the biofilm structure is completed, i.e. the biofilm can be treated as already matured, the conversion rate in the liquid flow is negligible in comparison with this one in the biofilm layers. This model shows that the phenomena considered as a part of overall bioprocess occurring in the both part of them are rather impressive by their complexity and mutual conditioning, which ensure methodological base to treated biofilms in the terms of the large complex systems [27]. Using this approach the large system, in our case - the biofilm, can be decomposed to subsystems for their more effective investigation. It also means lot of potentialities for creation of different types of partial cold or dead models, partial living (physiological) models and general living models as well as their mathematical descriptions and computer models with numerous possibilities of applications in the research and practical implementation. The system approach implementation in the biofilm R&D activity led us to the concept of the Bioprocess system as a notion reflecting the

unity of phenomena and processes not only in biofilm reactors, but in the field of bioreactor design and development of all types of the subjects of Bioprocess Engineering [4,28]. This approach can give a powerful impetus to start new more profound studies on the phenomena and effects having place in the biofilm for creating a more reliable experimental data bases for mathematical modeling of bioprocesses in biofilms [29].

The partial model is quite similar to the kinetic schemes of the heterogeneous catalysis with solid porous active masses in the Chemical Engineering. This analogy gives some advantages in the understanding the complexity of the processes in biofilms. However, being some kind of the dead photography of the part of phenomena in the bioprocess systems with fixed films, this model does not allow understanding the way of forming and functioning of biofilms as living structures. For this it is necessary to use another type of verbal model, which will reflect those typical for the living matter phenomena, that are in the roots of all the attributes of biofilms starting with the adaptation of the living cells to the new environment, their growth in liquid phase and on the solid surface, reproduction and, finally, their death and decay. The combination of this model with the above used will help here below understanding of the core of the problems in the biofilm formation and functioning and relief finding of the cross section of biotechnology with nanotechnology.

2. Biofilms as living self-organizing systems.

“State of the art” of the biofilms as self-organizing living systems

As a matter of fact, as first publication, in which it is mentioned the key word “biofilms” in combination with “self-organization”, can be recognized the paper of Caldwell [30]. The following 10 years are marked with quickly increasing interests, especially from the specialist of automatics, medicine and waste water treatment as typical areas of biofilm investigations. A statistics, made by us using the INTERNET search engine SCIRUS, shows that the period after is very fruitful with scientific production. The number of

publications on the biofilms as self-organizing systems up to 2008 is in general 70 (24 patents).

Verbal model of dynamics of biofilm formation and functioning.

For understanding the self-organizing character of the biofilms as living complex system a verbal model of its dynamics of initialization, forming and functioning is used here below. The fundamentals of this model have been depicted in 1990 [5]. It has been developed in following years by some modifications and introductions of new features [19,20,24]. The last version of the model consists of 7 consecutive stages, each of them is different from the foregoing by something new in the structure or in the expression of certain characteristic, like critical biofilm thicknesses – a parameter of crucial importance for mathematical forms of kinetics descriptions.

Admissions:

1. Biofilm formation and functioning are carried out in open systems.
2. The solid carriers are inert, i.e. they can not be substrates.
3. No mass- and heat transport limitations in the liquid phase.
4. Constant cell concentrations in the space of the liquid phase.
5. No cell aggregate formation in the liquid phase.

Stages of biofilm system development

The scheme of the model is presented in a chain of figures (Fig. 4a to 4f) which correspond to 7 stages of the biofilm formation and functioning. One can find the similarities or analogy with the thin layer formed by metals [31], for example - stages 1, 2, 3 and 4. The biofilm formation starts with stage 1 (Fig.1a). The main event here is the adaptation of cells suspended in the liquid phase, to the carrier. During this stage cells are “studying” the carrier surface for possible their attachment. In certain degree this process reminds the lag phase in the suspended cell cultures. In some cases, when the carrier is not suitable for colonization, they can “fabricate” and excrete special compounds, which afterward to be adsorbed on the carrier and to make its

surface familiar with them. The stage 1 is of high importance, especially for the aim of this study, because here is one of the cross point for convergence of biotechnology with nanotechnology. In dependence of the type of biofilm technology, the initializing of biofilm formation can be positive or negative event.

For the processing biofilm technologies it very important to have suitable carriers for biofilm formations, i.e. the time of adaptation of swimming cells is desirable to be as short as possible. This can decrease the period of biofilm formation and increase sensitively the effectiveness of the industrial bioprocess systems with fixed films. When the carrier is of natural origin, due to the long period of evolution of microorganisms, they are apt to recognize the “friendly” surface of the carrier and the initialization of the biofilm formation starts rather quick. In some cases, when the carrier is artificially synthesized, like when the polystyrene is selected as carrier, the time of initializing could prove to be inadmissibly long from technical and economical point of views. A very simple procedure, invented and developed by us for both laboratory scale [4] and implementations under industrial conditions, allowed to cut down substantially the duration of stage 1. The main manipulation in this procedure is the treatment of the carrier surface by powder of material to which the microorganisms in question have affinity, i.e. they are “acquainted” with its surface. For the microbial societies from waste water treatment stations it could be active carbon or coal. This material, named “promoter” by us, has to be drifted on the carrier surface by means of super speed mixer. The following operation includes multifold vigorous rinsing with tap water for washing out those powder particles, which have not been strongly linked with the carrier surface. The observation of such promoted surface shows different points consisted of clusters formed by the promoter and the surface layers of the carrier. It is quite possible these clusters to contain the formations of nano particles. The existence of such structures are to be proved, however it could be expectable that the conditions of the manipulations, especially high temperatures taking rise during vigorous mixing, can provide mutual penetration of the powder

and polystyrene, due to the co-melting of these two materials in the point of their contact. The application of this procedure firstly to polystyrene with active carbon and subsequently to many different carriers with various promoters for varied microorganisms and microbial societies always gave sensitive advantages. In connection with the development of the potentialities of nanotechnology it is quite possible that this primitive procedure can be improved or even radically changed by more sophisticated and effective method for preparation of carrier surface for swimming cells attachment. For this, it is necessary to detect those compounds that the cells produce when the carrier surface is not familiar with corresponding microorganisms. This example shows that it is possible by appropriate combination of some manipulations to give as a result imposing advantages in the field that can be common both for the biotechnology and nanotechnology.

As to protective biofilm technology the stage 1 is of very high importance too, because in accordance with its main aim it is impermissible even to allow starting of initialization of biofilm formation. Taking into account the biofilm the knowledge about biofilms, it means that for preserving the solid material it is inadmissible to let even one of the cells to be attached on its surface. Analysis of the literature shows that there are not suitable methods for absolute protecting of solid surfaces from cell attachment. Namely here it is a very important possible point of convergence of nanotechnology with the biotechnology, seizing of opportunity of which will have extremely positive impact in many scientific fields and industrial practices. The settling of the problem of absolute protection of the solid surfaces for example will close up biocorrosion, liquidate the biofilm formation on the artificial organs channels, avoid the biofilm formations in the nano membranes making the fine filtration processes durable, cut down the prolongations of many diseases provoked by the biofilms on the human body's organs, make away the biofilms from water supplying systems etc.

As to stage 2, it is well-known convergence point for nano- and biotechnologies, usually

referred as good example of activity in a common territory. It concerns the optical and electronic means of observations and investigations of biofilm surface. As to microscopy it is well-known cross point. The atomic force microscope (AFM) and the Scanning Tunneling Microscope (STM) are two early versions of scanning probes that launched nanotechnology, that are entirely used in biofilm investigations.

The other possible point of convergence is the implementation of nanosensors for measurement of concentration profiles of substrates and products of the bioconversion in biofilms. For stage 3 it is very useful because of possibilities for exact determination of the first critical biofilm thickness δ_{cr1} as a the frontier, behind which the influence of internal diffusion on the bioprocess kinetics is not negligible. It can give more precision of experimental data and will allow a precise determination of the bioprocess pure rate in the biofilm. The role of nanosensors is very important in the stage 4 as well. In analogy with stage 3 here again these devices can make possible more exact the determination of the second critical biofilm thickness δ_{cr2} and in the same time measurements of substrates and products concentration profiles. Having the correct pure conversion rate from stage 3 and precise concentration profiles from stage 4, it is not difficult to obtain the authentic value of the effective diffusion coefficients of substrates and products in biofilms. The putting into laboratory practice of nanosensors in investigation of stages 5 and 6 for concentration profiles determination will have the same positive effect on determination of δ_{cr3} and for obtaining more reliable information for biofilm state as one of the most important characteristics of bioprocess systems monitoring [32]. Summarizing the nanosensors implementations in biofilm research it can be underlined the expectations of enormous progress in the very near future of common implementation of nano- and biotechnology as two scientific fields.

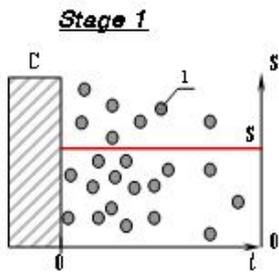


Fig.4a. Adaptation of cells to the carrier surface.

C – carrier; S – substrate; l – distance from the surface of the carrier; 1- swimming cells (suspended cell culture):

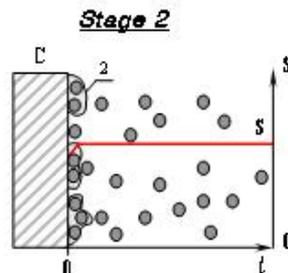


Fig.4b. Formation of cell monolayer.

2- adhered cells enveloped by exopolysaccharides

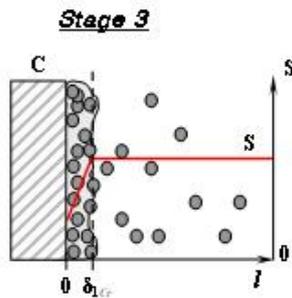


Fig.4c. Formation of the biofilm structure.

First critical thickness of the biofilm - δ_{1cr}

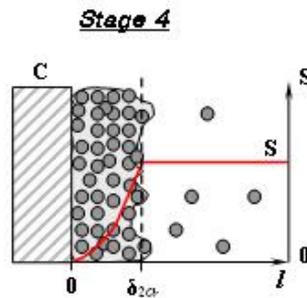


Fig.4d. Stable biofilm growth.

Second critical biofilm thickness - δ_{2cr}

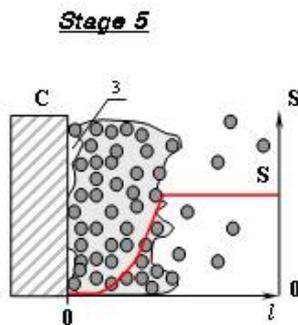


Fig.4e. Uncontrolled and unstable biofilm growth.

Cavities formation. 3 – cavity.

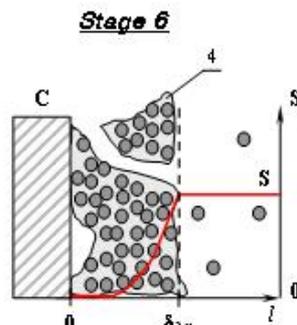


Fig.4f. Biofilm destruction.

Third critical biofilm thickness- δ_{3cr}

4 – Detached part of the biofilm structure

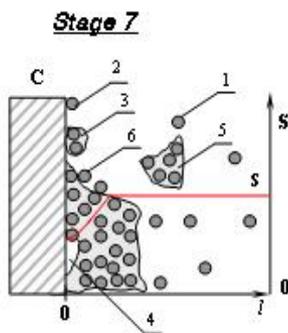


Fig.4g. Restart of new biofilm formation.

6 – new attached cells

During fixed periods of time, corresponding to every one of these 7 stages, in the biofilm proceed a lot of different processes, the roots of which are in physics, physical chemistry or biology. These stages reflect the results of accomplishment of main combinations of numerous phenomena of different nature, which set up the famous "spontaneous fixation" [5]. It is interesting to know, that the deals and roles of the phenomena-participants in the respective combinations for each of the stages are changing in the course of the biofilm formation and functioning. Thus, during the first stages physical and physico-chemical phenomena role is prevailing (stages 1 and 2), then in the latest parts of the biofilm formation course the biological phenomena predominate (stages 3 to 6). The final result of biofilm system dynamics development is shown on the Fig. 4f, that in fact present the "steady" state of the biofilm existence under the conditions of continuous of cultivation. It can be seen that this final stage includes all the previous ones (Fig.4a to 4e), but this stage is determined mainly by biological phenomena, because in fact there is not intact solid surface regarding cell influence. The new cells attach to the place, where their predecessors – the dyed cells swept from the carrier surface, have already been.

Looking for points of convergence from the biological positions, which give this last stage, it can be seen that the biofilms as complex self-organizing living systems, should be common territory both for biotechnology with its DNA techniques and nanotechnology with its methods in nano scale size for research and development activity. Using these finest methods in nano scale it will be possible to reveal the intrinsic and most intimate mechanisms of interactions between the cells in biofilm structure in each of the stages of its development and functioning. This will be of high importance for the realization of these systems enlarging the boundaries of applications in processing biofilm technologies by implementation of biofilms in fine microbial synthesis rationalizing the production of very effective medical remedies and pharmaceuticals.

In the same time, serious break-through can be expected in the biofilm protecting technologies. It is quite possible in near future

to find appropriate nanostructures, the synthesis of which on the artificial organs will repel the microorganism from their surface and will make impossible formation of biofilms. The deep understanding of the mechanisms of biofilm formation in potable water supplying systems, due to nanotechnology devices and methods will help finding of new remedies for avoiding the biofilm wall growth.

The list of future realization of converged bio- and nano- technology can be extended by applications in the field of biofuel cells, where the biofilm technologies have a key position [33].

CONCLUSION

The implementation of system approach in combination with the conception of self-organization allows development of verbal models of biofilms structures and dynamics of their formation and functioning. On this basis, the mechanisms of the main processes which occur in these cell formations are discussed. Using this knowledge and current terminology, some points of convergence of biotechnology with nanotechnology are outlined and several examples on this matter are given. Some spectacular future implementations as results of convergence of biotechnology with nanotechnology in R&D activity are outlined as well. The analysis of new notions reflecting possibilities to express the unification of the both fields – biotechnology and nanotechnology, in accordance with the information and discussed results, our own knowledge and experience in biofilm technology show that the convergence is better to be expressed by the notion of *nanobiotechnology*. Thus, along with the reaching the aim of this study, the field of convergence of these two most advanced branches of the modern R&D activity is determined to be related more to nanobiotechnology, then to bionanotechnology.

REFERENCES

1. Costerton J.W., Geesey G.G., Cheng K.J. (1978) How the bacteria stick, *Sci. Amer.*, v. 238, 137-144.
2. Melo L.F., T.R. Bott, M. Fletcher, Capdeville, B. (1992) Foreword to the NATO Advanced Courses on Biofilms, *Biofilms: Science and Technology* (eds.

L.F.Melo, B. Capdevill, M. Fletcher), NATO ASI Series, Series E: Applied science, Kluwer Academic Publishers, 223, 511-521.

3. Nikolov L., V. Mamatarkova, K. Ivanova (2003) Express method for assessment of the research trend in bioprocess engineering, *Annuaire de L'Universite de Sofia "St. Kliment ohridski", Livre 4 - 10^{eme} Session Scientifique, Sofia' 03*, 427-435.

4. Nikolov L., Karamanev, D. (1987) Experimental Study of the Inverse Fluidized Bed Bioreactor, *The Canadian Journal of Chem. Eng.*, v.65, 214-217.

5. Nikolov L., Karamanev D. (1990) The Inverse Fluidized Bed Biofilm Reactor - A New Tool for Biofilm Investigations, in the book: *Physiology of Immobilized Cells* (ed.J.A.M. DeBont et al.), Elsevier, 649-660.

6. Nikolov L. (1992) Properties and Application of Thiobacillus ferrooxidans Biofilms, in the book: *Biofilms: Science and Technology*, Kluwer Academic Publishers (ed. L.F. Melo et al.), NATO ASI Series, Ser. E: App. Sci. - vol. 223, 511-521.

7. Nikolov L., Karamanev D., Mehochev D., Dimitrov D., Mamatarkova V. (2002) Properties of the Biofilm of Thiobacillus ferrooxidans Formed in Rotating Biological Contactor, *Biochem. Eng.J.*, vol.12, 43-48.

8. Nikolov L., Mamatarkova, V. (2005) Bioprocess systems with fixed film - the base for development of ecological friendly technologies, *Ecol. Eng, Env. Prot.*, No 3-4, 16-32.

9. Costerton J.W., Lappin-Scott H. (1995) Introduction to Microbial Biofilms, in the book: *Microbial Biofilms* (Lappin-Scott H. and Costerton J.W. eds.), Cambridge University Press, 1-11.

10. Nikolov L. (2007a) Microbial biofilms – two sides of the medal, Plenary lecture at the scientific session “60 Years the Stephan Angeloff Institute of Microbiology”, Program and abstracts, Sofia, March 2007, R16, 17.

11. Mozer A. (1988) *Bioprocess Technology*, Springer-Verlag, New York Inc.

12. Characklis W.G., K.C. Marshall (1989) in *Biofilms*, (ed. W. Characklis&K.Marshall), John Willey&Sons, 10-12.

13. Moo-Young M. (1985) (a) Preface, in book: *Comprehensive Biotechnology*, Perg. Press, Ox., NY, Tor., Toronto, Sydney, 1, ix-xvii.

14. Document of the United Nations (2008) *The Convention on Biological Diversity (Article 2. Use of Terms).1992*. Retrieved on February 6, 2008.

15. Feynman R. (1959) There's Plenty of Room at the Bottom, Plenary lecture at the Am. Phys. Soc. Meeting, Caltech, California, December 29.

16. Taniguchi N. (1974) On the Basic Concept of 'Nano-Technology', *Proc. Intl. Conf. Prod. London, Part II*.

17. Kahn J. (2006) *Nanotechnology*, National Geographic (June), 98-119.

18. Document of the European Community (2006), Decision No 1982/18.12.2006 of EP concerning the 7th Framework program of European Community for Research and Technological development and Demonstration activities (2007 - 2013), <http://cordis.europa.eu/fp7/>.

19. Nikolov L., Mamatarkova V. (2007) *Technologies for Fluid Treatment. Part 2. Technological Link of Pollutants Destruction. Interactions of the Destructive Process with the Apparatus and Operating Regime*, *Ecol. Eng, Env. Prot.*, No 1, 41-52.

20. Nikolov L., Mamatarkova V., Petrova E., Stoychev S. (2005) Basic Phenomena in Bioprocess Systems with Fixed Film on Inert Solid Supporting Beads. Part 2. New Data about Biofilm Growth and Functioning, *Ecol. Eng, Env. Prot.*, No 2, 33-44.

21. Nikolov L., Proevska E., Mehochev D., Dimitrov D. (1985) Investigations on the solubility of sediments formed by spontaneously fixed microorganisms of species of Thiobacillus ferrooxidans in mineral acids, *Biot. Biotechn. Eq.*, No.6, 42-46.

22. Nikolov L. (2007b) Biofilm technology – common territory of biotechnology and nano technology, plenary lecture at the National information day: “Nano-sciences, nano-technologies, materials and new productive technologies in 7FP of EC”, Plovdiv, February 23rd, 2007, www.nsfb.net/system/storage/INFONANOBIOP.ttt.

23. Mamatarkova V., Nikolov L., Karamanev D. (2002a) *Biofilms: Problems and Trends in Research Activity. Part I: Biofilm Carriers, Biotechnology and Biotechnological Equipment*, 16,1, 170-176.

24. Mamatarkova V., Nikolov L., Karamanev D. (2002b) *Biofilms: Problems and Trends in Research Activity. Part II: Structure and Processes in Biofilms and Their Surroundings, Biotechnology and Biotechnological Equipment*, 16, 2, 200-207.

25. Characklis W.G., Bakker R, Trulear M.G. (1985) Fundamental considerations on fixed film systems, in book: *Comprehensive biotechnology*, Perg. Press, 5, 945.962.

26. Characklis W.G., Wilderer P.A. (1989) Structure and function of biofilms, in book: *Structure and function of biofilms*, J. Wiley&Sons, 1989, 5-17.

27. Nikolov L. (2006) Initiative Role of System Approach in the Investigations of Bioprocess

