Reconversion of oily greases from waste waters with the help of biofilms

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Abstract

Present paper matches the thematic area regarding bioprocesses, environment rehabilitation and non-polluting processing, through the fact that it proposes the use of biofilms with lipolythic characteristics having the capacity to metabolize greases (especially oils), which emitted into environment form a film at water surface and impedes oxygenation and they have a toxic effect over this, uncountable in time.

Biofilms use waste substrates as energy sources, transforming them into self cellular mass, which may be. Through bioenergetic conversion of organic wastes in polluted water into self organic compounds it results a cell mass which can be superiorly valorised.

Concretization of this complex combination between the two biologic and technologic components is represented by the biofixing systems reactors designing.

Keywords: environment, pollution, greases, biofilms

Introduction

Theoretically, the microbial aggregation was used as study model, theoretical for biological phenomena such as the cell to cell interaction, development, differentiation, morphogenesis, sexuality, communication, recognition, regulation, cell membrane surface, macromolecular specificity and contact reaction. [1,7].

Biofilms formation was divided in several phases: transport of microorganisms on substrate surface, initial adhesion (which is reversible), irreversible linking of microorganisms through filamentous appendix and the last phase is growing, rapidly and concomitantly multiply of the irreversible linked microorganisms and out of which new cells are released and that will populate new spaces. In this maturity phase there are mono-strata cells linked only to substrate and cells linked to substrate but also between them, and they form micro-colonies and biofilms; sometimes there added large quantities of specific polymers having reticular or fibrous structure, which additionally links the biofilm. [3,7].

Transformations at the level of the cell cover and in transport systems make adherent microorganisms be profoundly pheno-typical different from the ones in suspension. [6]. Recent studies on this issue show that adhesion determines expression of σ factor which unrepressed a high number of genes which pheno-typically transforms the cell. [6,7].

Biofilms involve the fact that each component microorganism lives in a community which has a homeostasis and a primitive “circulatory system” and also a metabolic cooperation that make these sessile cells have a fundamentally changed reaction towards the planctonic form of the same species. This confer them increased resistance at environment stress factors [3,7].

Thus, pheno-typical or comportamental resistance to antimicrobial substances is determined by: substrate adherence and microorganisms aggregation between each other; secreted exopolymers which form matrix of biofilm that protects enclosed cells (hypotesis rejected at present by some authors); degrading enzymes produced by species forming biofilm; adherence itself which determines physiologic changes that explain resistance to antibiotics; e. g. it was determined on *Escherichia coli*, that in the resistance to antibiotics gene sub-expression intervenes which codifies Omp F porine and thus being blocked hydrophille antibiotics penetration in cellular periplasmatic space. Implanting and development of biofilm are processes programmed by microorganisms genome (approximately 40% of genes are involved in this programming) [5,9].

In this field, the establishment of biofilms composition and activity represents a scientific tendency and promising practice. For this there are used microbiological, biochemical methods and molecular biologic techniques (DNA extraction, spectro-photometric determination, electrophoresis and PCR), which allow the differentiation of biofixed microorganisms types and species. This top field regarding biofilms and their metabolic activities, which are more various and more efficient comparatively with the same microbial cells found in suspension, is based on its fabulous evolution of sciences and technologies in the last years and presents an extremely special interest at international level. Thus, an important role in the emergence and development of

new technologies is played by the synergy between some fields, like: nano-technologies, bio-technology, informatics technology, bio-medical sciences. [2, 10, 11].

Materials and Methods

For studying of optimal working parameters, a series of laboratory experiments have been initiated, and they comprise both researches involving selected bacterial strains like *Pseudomonas,* and spontaneous microorganisms which have been self selected based on medium composition.

The principle used in order to form biofilms in vitro was auto-selection. The ones which survived and participated at biofilm forming were those microorganisms corresponding to environmental conditions, existent at the moment.

Researches initiated for development of biofilm aimed at selecting and adapting some microorganisms able to use oily waste fats mixed with detergents, transforming them into biomass.

The growth of microorganisms in this medium was realised in aeration-recirculation conditions, on glass plates.

Open plants for cleaning waste waters allow the development of some spontaneous biofilms which are resistant to environmental changes. This option is less costly, lasts more, and it is more resistant to fluctuations that happen in waste water content (changes in oil content, the presence of detergents, thermal and pH shocks, etc.) [2,10].

Based on these studies, it was created a collection of microorganisms with lipolythic enzymatic activity.

To establish dominant microorganism types which form the biofilm, there were carried out isolations from 10^{-3}-10^{-7}dilutions on Luria Bertani medium (LB) (7329A - Acumedia Manufacturers) and they were incubated at 29^{0}C. There were drawn isolated and well developed colonies which were passed on inclined LB medium (7329A - Acumedia Manufacturers) in the same conditions. These strains were examined for the following properties: Gram character, absence of sporulation, morphologic character (slightly curved rods), and mobility by means of polar flagels. There were selected the strains which presented also other common physiological properties: aerobic metabolism, fermentation absence and capacity to develop on different organic substrates.

To establish genetic and biochemical characteristics of isolated and dominant strains, from biofilms developed in described conditions, there were made biochemical tests with the help of API 20 NE kit and genetic tests through PCR methods.

Results and Discussions

Out of four examined and finally selected strains, three of all belong to *Psudomonas putida* species and the fourth one belongs to *Pseudomonas stutzerii* species.

After a hundred days period in recirculation-aeration conditions at room temperature it was noticed the development of a film (fig. 1), composed from a microbial culture, with variable thickness. From this biofilm, fragments can easily separate and are going to populate other free spaces, such as the tank walls or newly introduced glass plates.

Figure 1.

After washing in a strong water jet, the support covered by biofilm suffered some changes through removal of biofilm excess cells, yet remaining an adherent basic layer, from the development of biofilm.

Biofilms obtained by described method were introduced into a medium with an initial COD between 14 713 – 14 890 mg O_2/dm^3.

After 30 days the COD reached a level between 14 860 – 14 960 mg O_2/dm^3, the average being 14 889 mg O_2/dm^3 and after 60 days the level of COD was measured between 361 – 369 mg O_2/dm^3 for an average of 366 mg O_2/dm^3 CCOCr.

The above-mentioned data is presented in the table 1.

Table 1. Chemical Oxygen Demand (COD) in medium of oily waste water

No.	Sample	Initial mg O_2/dm^3	After 30 days mg O_2/dm^3	After 60 days mg O_2/dm^3
1	S I	14.713	14.860	361
2	S II	14.890	14.893	368
3	S III	14.833	14.916	369

Biofilms obtained in the laboratory from selected strains are less resistant to environmental changes than those biofilms obtained in spontaneous conditions;

Considering that industrial units belonging to agro-food sectors (covered in table 4, annex 1-NTPA – 0,11), proper collecting and cleaning of industrial waste waters with COD of 500 mgO_2/dm^3 maximum admitted have to be observed for waste waters discharged in local sewage systems [8]. Our experiments revealed a reduction of COD from an average of 14.889 mg O_2/dm^3 to an average of 366 mg O_2/dm^3 CCOCr, fact that allows the evacuation in the sewage system (in conformity with MO part I no. 398/11.05.2005).

Conclusions

Treatment of waste waters with oily content, with the help of biofilm presents the following advantages:

- irreversibly linked microorganisms grow and rapidly multiply comparatively with the ones in suspension;
- biofilm is more active than cells in suspension; more resistant to environment variations and to stress factors generally;
- once formed, the biofilm has a long span activity even with nutrients in very small quantity;
- removal of biofilm upper layers leads to intensification of its activity (respectively biomass can be superiorly valorised.).

Experiments conducted in laboratory highlighted data that confirm information from specialized literature.

References

1. ALVES M. M., MOTA VIEIRA J. A., ALVARES PEREIRA R. M., PEREIRA M. M., MOTA M.– Effect of lipids and oleic acid on biomass development in anaerobic fixed-bed reactors. Part I: Biofilm growth and activity. Wat. Res., vol. **35**, No 1 pp. 255-263, 2001
2. BERTIN L., MAJONE M., GIOIA G., FAVA F. – An aerobic fixed-phase biofilm reactor system for the degradation of the low-molecular weight aromatic compounds occurring in the effluents of anaerobic digestors treating olive mill wastewaters. Journal of Biotechnology **87**, 161-177, 2001
3. BUSSCHER H. J., BOS, R., van der MEI, H. C. – Initial microbial adhesion is a determinant for the strength of biofilm adhesion. FEMS, Microbiology Letters, **128**: 229 – 234, 1995
4. BRUCE C. ALLEMAN, BRUCE E. LOGAN, ROBERT L. GILBERTSON. – Degradation of pentachlorophenol by fixed films of white rot fungi in rotating tube bioreactors. Wat. Res., vol. **29**, No 1, pp. 61-67, 1995
5. COSTERTON, J. W., LAPPIN – SCOTT, H. M. – Behaviar of bacteria in biofilms. ASM News, **55**: 650-654, 1989
6. HAMILTON W. A., CHARACKLIS, W. G. – Relative activities of cells in suspension and biofilms. In "Structure and function of biofilms" New York, Dahlem Konferenzen, 200-210, 1993

7. LAZĂR V.– Aderența microbiană, Ed. Academiei Române, 2003
8. MO part I no. 398/11.05.2005
9. QINGWEI LIU, KAREN M. MANCL, OLLI H. TUOVINEN – Effect of inoculation on the biodegradation of butterfat-detergent mixtures in fixed-film sand columns. Bioresource Technology, vol. **64**, pp. 27-32, 1998
10. RAY P., OUBELLI M., LÖSER C. – Aerobic 4-nitrophenol degradation by microorganisms fixed in a continuously working aerated solid-bed reactor. Appl. Microbiol. Biotechnol., **51**, pp. 284-290, 1999
11. COLON PAUL – La chimie verte. Editions Tec&Doc, Lavoisier, Biodégradabilité, **17**, pp. 487-509, 2006