Récents Progrès en Génie des Procédés, Numéro 104 - 2013
ISSN: 1775-335X ; ISBN: 978-2-910239-78-7, Ed. SFGP, Paris, France

Biofilm formation on metal structured packing for the production of high added value biomolecules

Zune Quentin[a*], Brognaux Alison[b], Ongena Marc[c], Toye Dominique[d], Thonart Philippe[b] ,Delvigne Frank[c]

[a]Uni. Liege-Gembloux Agro-Bio Tech. Unité des Bio-Industries. Thèse financée par le FRIA
Passage des déportés, 2. B-5030 Gembloux (Belgium)

[b]Univ. Liege-Gembloux Agro-Bio Tech. Unité des Bio-Industries.
Passage des déportés, 2. B-5030 Gembloux (Belgium)

[c]Univ. Liege-Gembloux Agro-Bio Tech. FRS-FNRS Unité des Bio-Industries.
Passage des déportés, 2. B-5030 Gembloux (Belgium)

[d]Univ. Liege. Chemical Engineering Laboratory.
Allée de la Chimie, 3/6c. B-4000 Liege (Belgium)

Abstract

Many white biotechnology bioprocesses apply techniques from chemical engineering based on bioreactors with mechanical stirring system commonly employed in pharmaceutical sector, food industry or energy field (Dasilva, 2004). As in chemical engineering, scale-up of these bioprocesses induces physicochemical constraints that affect physiological pathways and decrease performances. In this context, it is essential to think new bioprocesses better suited to physiology of microorganisms, minimizing physicochemical constraints. The aim of this work consists to use stainless steel structured packing (*SSP*) with high specific area (500-750 m^2/m^3) as inert support for biomass immobilization in order to produce high added value biomolecules. These bioreactors are biocatalysts in which microbial system is immobilized biomass on the form of a biofilm performing bioconversion of a substrate into a specific product (Rosche, 2009). In this study, an experimental setting containing a *SSP* reproduces solid-state fermentation (*SSF*) like conditions. Two well known microorganisms for their ability to form biofilm and secrete metabolites are tested in the experimental setting : *Bacillus subtilis* for its lipopeptides and *Aspergillus oryzae* for its glucoamylase. Effectiveness of the bioprocess in term of dynamic of the excretion of the target biomolecule is compared with a classical submerged culture (*SmF*). For lipopeptides production from *B. subtilis*, *SSP* is located in a 20L bioreactor continuously aspersed by liquid medium required to the growth of the biofilm. In the case of *A. oryzae, t*he *SSP* is partially immerged in a 250 mL shake flask. X-ray tomography of the *SSP* allows non-invasive visualization and quantification of biofilm repartition inside the support.

Implementation of *SSP* permits almost total immobilization of biomass on the form of a mono-species biofilm to the detriment of the liquid phase. Processing of images obtained by X-ray tomography of the *SSP* provides relevant information for the optimization of the bioprocess. For both microorganism species, results indicate the influence of parameters such as hydrodynamics, aeration rate and microorganism specificity, on the biofilm morphology inside the support and the performances of the bioprocess. *SSF*-like conditions in the experimental setting lead to technologic progress, such as absence of foam formation, persistence of the microbial system, and improve the dynamic of metabolites excretion compared with conditions imposed by the submerged culture. Further experiment will consider hydrodynamics aspects and amount of carbon source on effectiveness of the bioprocess.

Keywords : biofilm reactor, biomolecule, stainless steel structured packing, *B. subtilis*, *A. oryzae*

* corresponding author: *E-mail address* : qzune@doct.ulg.ac.be

Récents Progrès en Génie des Procédés, Numéro 104 - 2013
ISSN: 1775-335X ; ISBN: 978-2-910239-78-7, Ed. SFGP, Paris, France

1. Introduction

Most of bioprocesses developed for environmental applications involve immobilized microbial systems on inert or organic supports on the form of a biofilm (Qureschi, 2006). In these bioreactors, biofilms are organized cells consortiums (multiple-species biofilms) assimilated as biocatalysts of pollutant molecules. Despite inimical environmental conditions met in these bioprocesses, biofilms tolerate and adapt without alter good performances of bioconversion. Nowadays, single-species biofilm reactors have been investigated for low added value operations such as acetic acid or ethanol production (Cheng, 2010). Major part of bioprocesses involved into pharmaceutical applications or food industry are performed in pure culture carried out in stirred tank bioreactor. However, scale-up of these bioprocesses leads to physico-chemical constraints affecting physiology of free biomass and decreases bioprocess performances (Delvigne, 2010). In this context, it is required to design new bioreactors better suited to physiology of microorganism and less influenced by process intensification.

Purpose of this work is to design a new model of bioreactor based on environmental bioprocess for the production of high added value biomolecules. Such bioprocess is attractive for microbial strains forming biofilms easily and secreting a broad spectrum of metabolites in the environment (Rosche, 2009, Babu Halan, 2012). In this kind of bioreactor, growth of the microbial system on the form of a mono-species biofilm immobilized on a inert support reproduces solid-state fermentation (*SSF)* like conditions (Barrios-González, 2012). The experimental setting developed for the study contains a metal structured packing (Sulzer, Chemtech) as inert support for the immobilization of the biomass on the form of a biofilm. The stainless steel support is composed by assembly of staggered corrugated sheets in order to maximise area density (750 m^2/m^3) required for good exchanges between biofilm, liquid and gas phase. Nutrients supply is performed by percolation of the medium on the metal corrugated sheets of the packing thanks to a recirculation pump. The aim of this study is to compare effectiveness of a bioprocess reproducing *SSF*-like conditions, such as those encountered in a biofilm reactor, with a conventional submerged culture. Several parameters as kinetic of carbon consumption, production of the biomolecule of interest, biofilm distribution on the support are measured in order to characterize the bioprocess and to compare its performances with a classical submerged culture (*SmF*). X-ray tomography is a relevant tool for biofilm visualization and quantification inside the support. This technique allows to understand how parameters as hydrodynamics or microbial strain affect performances of the bioprocess. This experiment is carried out with two species of microorganism known for their abilities to form biofilm and to secrete biomolecules : *Bacillus subtilis* for the production of lipopeptides (surface-active biomolecules) and *Aspergillus oryzae* for the production of glucoamylase (an amylolytic enzyme).

2. Materials and Methods

Microbial strain and medium

Bacillus subtilis GA1 strain, stored in working seed vials at -80°C, was used for lipopeptides production. Precultures and cultures were performed on a optimized medium containing (saccharose 20 g L^{-1}, pepton of casein 30 g L^{-1}, yeast extract 7 g L^{-1}, KH_2PO_4 1,9 g L^{-1}, $MgSO_4$ 0,45 g L^{-1}, citric acid 10 mg L^{-1}, solution 1 100 µL, solution 2 100 µL). Solution 1 (pH 7) is composed of H_3BO_3 100 mg L^{-1}, $NaMoO_4$ 40 mg L^{-1}, $FeCl_3.6H_2O$ 50 mg L^{-1}, KI 20 mg L^{-1} and $CuSO_4$ 10 mg L^{-1}. Solution 2 (pH 7) is composed of $MnSO_4.H_2O$ 36 g L^{-1} and $ZnSO_4.7H_2O$ 140 mg L^{-1}. Before operating in the bioreactor, an overnight precultivation step is performed in 200 mL of the above-mentioned medium in shake flask at 37°C.

Aspergillus oryzae ATCC16868 strain used for glucoamylase production carries a fluorescent reporter system specifically expressed in solid-state fermentation (*SSF*) like conditions, i.e. in the conditions of the experimental setting used in this work. The glucoamylase B gene (*glaB*) was shown to be highly expressed during solid state cultivation (Ishida H, 2006). Then, an expression vector based on *glaB* expression signals was generated and inserted into the genome of *A. oryzae* ATCC 16868. The expression vector is composed of the promoter region of the *glaB* gene linked to a gene sequence (*gla::gfp* fusion gene derived from an expression vector described by Gordon et al. (2000)) carrying the encoding gene of the green fluorescent protein (*gfp*) as marker gene to monitor *glaB* expression. Previous studies with similar reporter system showed that quantification of the gene of interest can be estimated by quantifying

the amount of GFP (Delvigne, 2011). Petri dish with PDA medium was inoculated with fungal spores and incubated for 1 week at 30°C and stored at 4°C for up 2 weeks before harvest by addition of 9 mL of peptone water and scraping with a sterile syringe needle. The spores from one petri dish were counted using a Burker cell in order to inoculate the liquid medium (soluble starch 5 g L^{-1}, casein peptone 5 g L^{-1}, yeast extract 5 g L^{-1}, chloramphenicol 400 mg L^{-1}, pH 6) to the target density of 2-5 x 10^8 spores / L.

Operating procedures for solid-state and submerged culture

For each strain, fermentation run is performed in an experimental setting (Figure 1) that reproduces conditions of solid-state fermentation (*SSF*) and is compared with a classical submerged culture (*SmF*). Samples collected during fermentation are filtered in order to recover the supernatant. These latter are stored at 4°C before further analysis. Fermentation runs are performed on period of 72 hours.

The experimental setting used for lipopeptides production by *B. subtilis* GA1 is a 20 L tank containing 6 L of liquid optimized medium above which was located a stainless steel structured packing (*SSP*) (Sulzer, Chemtech) (Figure 1A, 1B and 1C). A peristaltic pump ensured medium recirculation at a flow rate of 26 L h^{-1} in a silicone tubing which connects the bottom to the top of the reactor. Air supply was injected under the packing at a flow rate of 1 *vvm*. The air injection system was located above the liquid surface in order to avoid foam formation during lipopeptides production. The submerged culture is carried out in a 20L stirred tank reactor containing 14 L of liquid optimized medium with 200 µL/L of antifoam. Inoculation was made with 2% (v/v) of the overnight preculture and temperature and pH are maintained respectively at 37°C and 6.95 for each run.

The experimental setting used for glucoamylase production by *Asp. oryzae* is a 20L tank containing a *SSP* totally immersed in 16L of liquid medium (Figure 1D). Mechanical stirring axis has been removed and air supply is provided in the bottom of the tank at a flow rate of 1 vvm. Temperature and pH are respectively maintained 30°C and 6 during culture. Inoculation is performed with the same density of spores (2-5 x 10^8 spores / L) from a same petri dish.

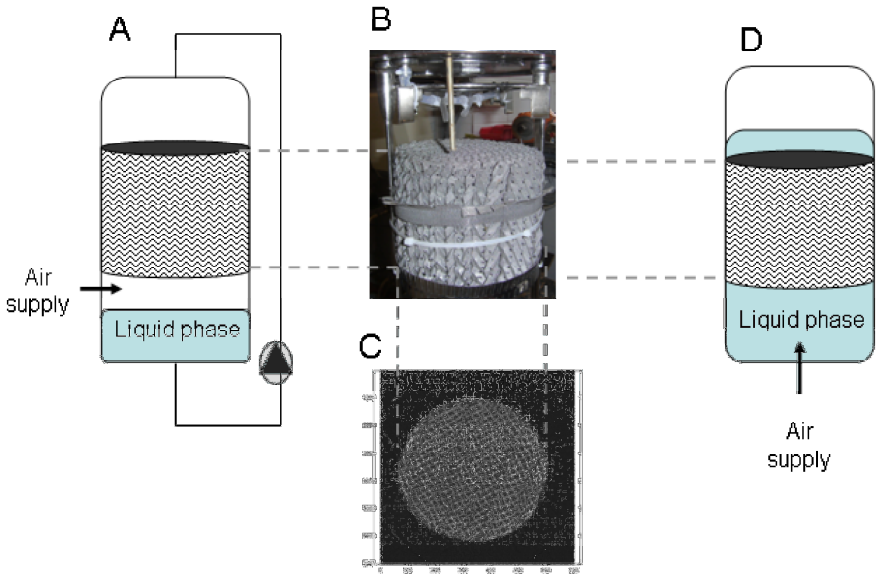

Figure 1 : Design of the experimental setting used for SSF-like conditions. A : Scheme of the bioreactor used for B. subtilis fermentation. B : stainless steel structured packing (SSP). C : X-ray tomography picture of a cross sectional area of the packing. D : Scheme of the bioreactor used for A. oryzae fermentation.

X-ray tomography analysis

After 72 hours of fermentation, *SSP* is removed from the experimental setting and drained for 2 hours in order to remove excess liquid medium before X-ray tomography analysis. This latter is non-invasive

technique allowing visualization and quantification of the biofilm structure inside the support. Collected data from the X-ray tomography permits the reconstruction of an image in two dimensions corresponding to a given cross-sectional area of the *SSP*. Tomographic measurements were performed on 16 packing cross-sectional area located at different heights (every centimeters). Then, image processing consists to remove pixels corresponding to the metal corrugated sheets and allows the quantification of the surface effectively occupied by biofilm over different packing cross-sectional area. Experimental method for tomographic measurements and subsequent treatment for absorption coefficient processing and image analysis have been previously described by (Aferka, 2011).

Lipopeptides quantification in the culture supernatant

Supernatant of samples collected during fermentation were analyzed by reverse phase HPLC coupled with single quad mass spectrometer (HPLC Waters Alliance 2695/diode array detector, coupled with Waters SQD mass analyzer) on a X-terra MS (Waters) 150*2.1 mm, 3.5 µm column as previously described by (Nihorimbere, 2011).The elution gradient allows the simultaneous measurement of all three lipopeptides families : surfactin, fengycin and iturin. Compounds were first identified on the basis of their retention times compared to purified standards and the amounts were calculated on the basis of the corresponding peak area (maximum plot). Homologues of each family were identified on the basis of their mass spectra detected in the mass spectrometer.

Gluco-amylase assay in the culture supernatant

As mentioned above, glucoamylase secreted by *A. oryzae* can be estimated by quantifying amount of the green fluorescent protein (*GFP*) in the extracellular medium. Quantification of *GFP* is assessed by immunoblot analysis in order to consider the totality of *GFP* found in the culture supernatant. Indeed, instable *GFP* does not emit fluorescence and is not consider in the case of an analysis by spectrofluorimetry. Band intensity can be measured with the software Bio1D and is proportional to *GFP* concentration. A positive control performed with the wild strain of *A. oryzae* permits to reveal the reliability of the reporter system as a physiological tracer of the bioprocess effectiveness.

3. Results and Discussion

Fermentation run with *B. subtilis* GA1

X-ray tomography analysis

X-ray tomography analysis of seven cross-sectional area of the metal packing are given in the figure 2A and 2B. The three-dimensional biofilm structure exhibits a conical shape starting from the top of the packing. This result suggests that liquid repartition over the whole cross-sectional area of the packing is not effective despite the presence of a liquid distributor. Clogging induced by the thick matrix of the biofilm appears between corrugated sheets of the *SSP* and leads to the fall of biofilm fragments into the liquid phase. Biomass attached to the support represents 93 % of the total biomass, the remainder being in the liquid phase.

Comparison of lipopeptides production between the biofilm reactor and the stirred tank reactor

Lipopeptides synthesized by *B. subtilis* include several classes of high-valued molecules with surface-active and antibiotic properties. Qualitative comparison of lipopeptides produced under the two conditions is given in the figure 2C. This latter shows ratio of peaks area from surfactin, fengycin and iturin families between the two bioreactor operating conditions. Fengycin and iturin contents are higher in the case of the stirred tank reactor but surfactin contents is 1,25 times higher in the biofilm reactor.

Our attention has been mainly focused on the dynamic of surfactin production since it represents the main class of lipopeptides found at the end of the cultures (Figure 2D). Final concentration of surfactin obtained in the fermentation broth after 72 hours of culture are respectively 345,4 ± 32,8 mg / L and 277,3 ± 34,4 mg / L for the biofilm reactor and stirred tank reactor. Surfactin concentration is still increased if the amount of surfactin trapped in the matrix of the biofilm is taken into account. Indeed, about 20% of the total amount of surfactin is contained in the extracellular matrix of the biofilm at the end of the culture (results not shown).

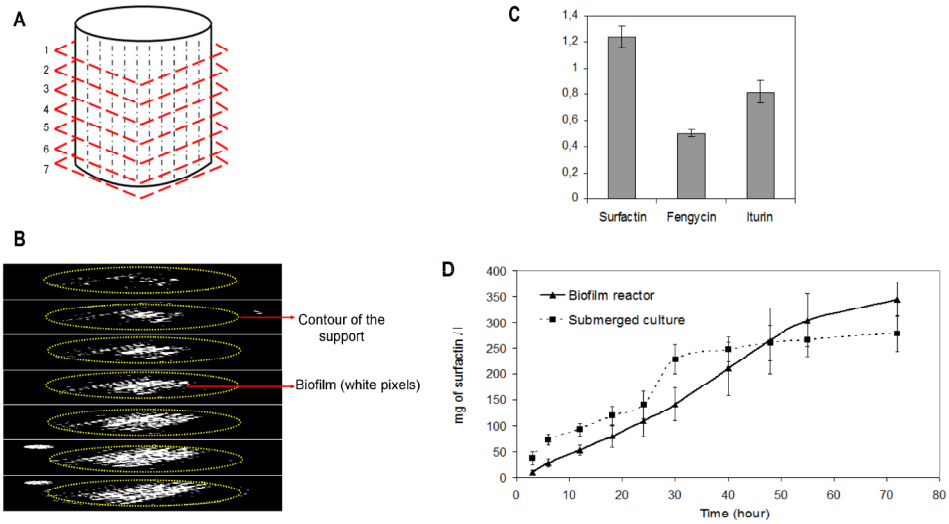

Figure 2 : A and B : Scheme and image stacking from X-ray tomography analysis of seven cross-sectional area of the metal packing. C : Ratio of peaks area from surfactin, fengycin and iturin families between the SSF and the SmF after 72 hours of fermentation. D : Comparison of the surfactin (mg / L) production during fermentation in the culture medium of the biofilm reactor and the submerged culture..

Discussion

Despite low differences between the surfactin concentrations for the two operating conditions (1,25 times more in the *SSF*-like conditions), the design of the experimental setting leads to a technological progress because it avoids foam formation and does not require addition of an antifoaming agent. Indeed, major drawback of aerated and stirred bioreactors usually used for lipopeptides production is the large foam formation decreasing oxygen transfer rate and increasing the risks of contamination. Addition of an antifoaming agent limits foam formation but leads to difficulties during downstream processing operations. Solid-state conditions seem to be well suited for bioprocess involving foam formation. Nevertheless, from an effectiveness point of view, biofilm repartition inside the support needs to be optimized as a thin layer in order to avoid clogging and to improve mass transfer of nutrients/metabolites inside the biofilm matrix.

Fermentation run with *A. oryzae*

X-ray tomography analysis

During the culture in SSF-like conditions, immerged *SSP* is rapidly and entirely colonized by the mold. The image processing of a X-ray tomography analysis of a cross sectional area confirms this observation (Figure 3A). The extension of this latter shows a constant layer of mycelium overlaying all the surface of the corrugated sheets without causing clogging (Figure 3B). Consequently, voids allow good exchanges between the mold and the liquid phase. The fermentation broth is perfectly limpid during all the culture because all the biomass is attached on the support.

Comparison of GLA::GFP production between the biofilm reactor and the stirred tank bioreactor

The *glaB* expression is normally observed on a larger extent in SSF by comparison with SmF where *glaB* is less active (Ishida H, 2006). In SSF conditions, *GLAB* protein is excreted in the extracellular medium in order to hydrolyze non-reducing ends of starch in ß-glucose, a digestible carbon source for the mold. Previous experiment demonstrates the reliability of the reporter system to be used as a physiological tracer to assess the efficiency of the process (results not shown) and defines the molecular weight of the *GLA::GFP* fusion protein secreted in the culture supernatant at 70 kDa (results not shown). At different time of culture, an immunoblot analysis of the extracellular medium (Figure 3C) is carried out in order to take into account degraded or denatured *GLA::GFP*. The detection with an anti-GFP antibody reveals the presence of *GLA::GFP* fusion protein only in the culture supernatant of the biofilm reactor. This result

highlights the difference between both bioprocesses and is supported by the fact that SSF-like conditions promotes the excretion of metabolites (Barrios-González, 2012). An other band appears at a molecular weight of 27 kDa and could correspond to a proteolytically truncated *GFP*. Relative abundance of the *GLA::GFP* fusion protein during SSF culture (Fig 3D) shows an increase from the 42nd hour with a maxima at the 58th hour. Protein reconsumption by the mold could possibly explain the decrease of the *GLA::GFP* fusion protein at the end of the fermentation.

4. Discussion

Bioprocess conditions of the submerged culture in the stirred tank reactor and the semi-solid culture in the experimental setting have a different impact on physiology of the mold and the performances of the bioprocess. The great shear forces caused by mechanical stirring in the submerged culture induce mold growth in the form of a free mycelium involving an increase of medium viscosity. In the semi-solid culture, mycelium colonizes the *SSP* by forming a thick layer of biomass and allows for high expression of *glaB* compared with the submerged culture. Air supply in the bottom of the tank acts as agitation of the liquid medium like in a bubble column. Maintaining a liquid phase free of biomass during all the culture improves dynamic of bioprocess and could simplify downstream processing operations. Nevertheless, thickness of the mycelium should be controlled in order to optimize mass transfer of the *GLAB* from the support to the liquid phase.

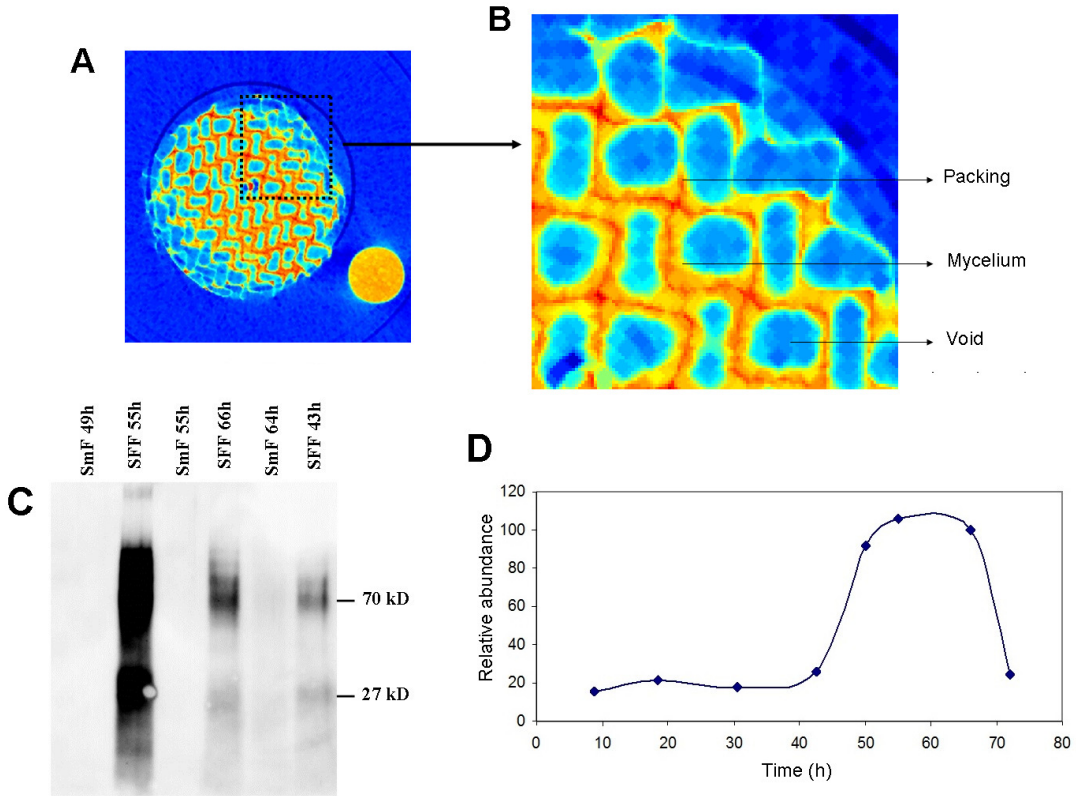

Figure 3 : A : Processed image highlighting biofilm from raw tomographic data of a cross sectional area of the metal packing. B : Extension of A in order to focus on biofilm colonization in the metal packing. C : Immunoblot analysis of GLA::GFP excretion in the supernatant of cultures under SSF and SmF conditions. D : Kinetic of GLA::GFP excretion in the supernatant of SSF culture in the 20L bioreactor.

4 Conclusions

In this study, an experimental setting reproducing conditions of solid-state fermentation was used for high added value metabolites production and was compared with a classical submerged culture. The experimental setting contains a metal structured packing allowing microbial growth as a biofilm. *SSF*-like conditions lead to technologic progress such as absence of foam formation, persistence of the microbial system and improvement of downstream processing operations. Moreover, dynamic of metabolites excretion is improved in *SSF*-like conditions but are not yet optimized. Consequently, it is required to define physicochemical and biological parameters affecting the biofilm distribution inside the structured packing and the excretion of the metabolites. X-ray tomography proved to be a relevant tool to characterize parameters influencing microbial growth inside the support. The next step of this work will consider hydrodynamics aspects such as liquid and gaz flow rate and physiologic aspects such as amount and origin of the carbon source.

Acknowledgments

Quentin Zune is supported by a FRIA PhD grant from the Belgian Fund for Scientific research (FNRS). All the authors gratefully acknowledge Peter J. Punt (*Wageningen Center for Food Science*) for the construction of the strain *Aspergillus oryzae* ATCC 16868 with the fluorescent reporter system used in this work.

5. References

AFERKA, S., VIVA, A., BRUNAZZI, E., MARCHOT, P., CRINE, M., TOYE, D. 2011. Tomographic measurement of liquid hold-up and effective interfacial area distributions in a column packed with high performance structured packings. *Chemical engineering science,* 66**,** 3413-3422.

BABU HALAN, K. B. A. A. S. 2012. Biofilms as living catalysts in continuous chemical syntheses. *Trends in Biotechnology,* 30**,** 453-465.

BARRIOS-GONZÁLEZ, J. 2012. Solid-state fermentation: Physiology of solid medium, its molecular basis and applications. *Process biochemistry,* 47**,** 175-185.

CHENG, K. C., DEMIRCI, A., CATCHMARCK, J.M., 2010. Advances in biofilm reactors for production of value-added products. *Applied microbiology and biotechnology,* 87**,** 445-456.

DASILVA, E. J. 2004. The colors of biotechnology: Science, development and humankind. *Electronic Journal of Biotechnology,* 7.

DELVIGNE, F., BROGNAUX, A., GORRET, N., SORENSEN, S.J., CRINE, M., THONART, P., 2011. Applicability of GFP microbial whole cell biosensors to bioreactor operations: mathematical modeling and related experimental tools. *Biosensors for Health, Environment and Biosecurity / Book 1*. Intech.

DELVIGNE, F., DESTAIN, J., BROGNAUX, A., KAR, T., LEJEUNE, A., THONART, P., 2010. L'extrapolation des bioréacteurs : un problème de génie des procédés ou de physiologie microbienne. *Chimie nouvelle,* 104**,** 7-14.

ISHIDA H, H. Y., KAWATO A, ABE Y. 2006. Improvement of the glaB promoter expressed in solid-state fermentation (SSF) of Aspergillus oryzae. *Bioscience, Biotechnology and Biochemistry,* 70**,** 1181-1187.

NIHORIMBERE, V., CAWOY, H., SEYER, A., BRUNELLE, A., THONART, P., ONGENA, M. 2011. Impact of rhizosphere factors on cyclic lipopeptide signature from the plant beneficial strain Bacillus amyloliquefaciens S499. *FEMS Microbiology Ecology,* 79**,** 176-191.

QURESCHI, N., ANNOUS, B.A., EZEJI, T.C., KARCHER, P., MADDOX, I.S., 2006. Biofilm reactors for industrial bioconversion processes : employing potential of enhanced reaction rates. *Microbial cell factories,* 4**,** 1-21.

ROSCHE, B., LI, X.Z., HAUER, B., SCHMID, A., BUEHLER, K., 2009. Microbial biofilms : a concept for industrial catalysis ? *Trends in biotechnology,* 27**,** 636-643.

Récents Progrès en Génie des Procédés, Numéro 104 - 2013
ISSN: 1775-335X ; ISBN: 978-2-910239-78-7, Ed. SFGP, Paris, France

Formation de biofilm sur garnissages métalliques structurés pour la production de biomolécules à hautes valeurs ajoutées

Quentin Zune*[(a)], Brognaux Alison[(b)], Marc Ongena[(c)], Dominique Toye[(d)], Philippe Thonart[(b)], Frank Delvigne[(c)]

[a] Univ. Liege- Gembloux Agro-Bio Tech. Bio-Industries Unit. Passage des Déportés, 2. B-5030 Gembloux (Belgium). Thèse financée par le FRIA.
[b] Univ. Liege- Gembloux Agro-Bio Tech. Bio-Industries Unit. Passage des Déportés, 2. B-5030 Gembloux (Belgium).
[c] Univ. Liege- Gembloux Agro-Bio Tech. F.R.S-FNRS. Bio-Industries Unit. Passage des Déportés, 2. B-5030 Gembloux (Belgium).
[d] Univ. Liege. Chemical Engineering Laboratory. Allée de la Chimie, 3/6c. B-4000 Liege (Belgium).

Résumé

De nombreux procédés microbiens utilisés dans les biotechnologies blanches ont recours aux techniques du génie chimique. Cela est notamment le cas des bioréacteurs à cuves agitées employés dans le secteur pharmaceutique, agro-alimentaire ou énergétique. A l'instar du génie chimique, l'extrapolation à l'échelle industrielle s'accompagne de contraintes physico-chimiques qui occasionnent des modifications physiologiques, affectant ainsi les performances du bioprocédé. Dans ce contexte, il est nécessaire de concevoir des bioprocédés mieux adaptés à la physiologie des microorganismes et qui minimisent les contraintes physico-chimiques. Inscrit dans cette thématique, cette étude vise à utiliser des garnissages métalliques structurés (*GMS*) à grande surface spécifique (500-750 m²/m³) en tant que supports inertes destinés à immobiliser des cellules microbiennes pour la production de biomolécules à hautes valeurs ajoutées. Ces modèles de bioréacteurs peuvent être assimilés à des biocatalyseurs dans lesquels la biomasse, immobilisée sous forme d'un biofilm microbien, catalyse la transformation d'un substrat en un produit. Dans ce travail, un dispositif expérimental contenant un *GMS* tente de reproduire les conditions d'une fermentation en milieu solide (*SSF*). Deux microorganismes connus pour leur capacité à former des biofilms et à sécréter des métabolites sont testés dans ce dispositif expérimental : *Bacillus subtilis* pour ses lipopeptides et *Aspergillus oryzae* pour la gluco-amylase. Les performances du système en terme de dynamique d'excrétion des métabolites d'intérêt sont comparées à une culture submergée classique (*SmF*). Le *GMS* est soit positionné dans un bioréacteur et aspergé en continu par du milieu de culture nécessaire à la croissance du biofilm dans le cas de *B. subtilis*, soit il est immergé partiellement dans une fiole agitée dans le cas d'*A. oryzae*. La colonisation du support par la biomasse microbienne est quantifiée et visualisée non-invasivement par tomographie à rayons-X du *GMS*. De la même manière que certains réacteurs à support utilisés en épuration des eaux, l'implémentation du *GMS* permet d'immobiliser près de la biomasse totale au détriment de la phase liquide. L'analyse des images obtenues par tomographie à rayon-X du *GMS* fournit des informations pertinentes pour l'optimisation du procédé. Les résultats obtenus pour les deux microorganismes indiquent que des paramètres tels que l'hydrodynamique du liquide, l'aération et le type de microorganisme influencent la distribution du biofilm. Les conditions de fermentation en milieu solide imposées par le dispositif expérimental mènent à des progrès technologiques, tels que l'absence de mousse et la rémanence du système microbien, et à une amélioration de la dynamique de sécrétion des métabolites par rapport aux conditions imposées par la culture submergée.

Mots-clés: biofilm, biomolécule, garnissage métallique structuré, *B subtilis, A. oryzae*

* auteur correspondant: *E-mail address* : qzune@doct.ulg.ac.be