УДК 577.15

INVERSE PHASE TRANSFER BIOCATALYSIS FOR A BIODESULFURIZATION PROCESS OF MIDDLE DISTILLATES

L. Setti, S. Bonoli, E. Badiali, S. Giuliani

(Department of Industrial Chemistry and Materials, V.le Risorgimento 4, I40136 Bologna; Lesepidado srl, V. G. Dozza, 5-I40025 Bologna)

Biocatalytic desulfurization of fuels presents some problem concerning the by-products inhibition of the biocatalysts and diffusive mechanisms between the organic and the aqueous phases. The inverse phase transfer biocatalysis (IPTB) is based on the use of supramolecolar receptors (modified cyclodextrines), which selectively pick-up the sulfur aromatic compounds in the organic phase and transfer them into the water phase where the biocatalyst is located. It can both improve the mass transfer of water insoluble substrates between organic and aqueous phases and decrease and/or eliminate the feed-back inhibition of the biocatalyst due to the by-products accumulation in water phase.

Biocatalytic desulfurization of fuels is still not a commercial technology because some problem concerning both the conversion and finishing which are the critical steps of any biorefining process in petrochemistry have to be solved. Conversion and finishing are affected by a number of crucial factors, namely: 1) biocatalyst specificity; 2) biocatalyst stability; 3) biocatalyst activity; and, 4) bioreactor design, in which the volumetric ratio between the oil phase and the aqueous medium represents the main limiting factor for an industrial application of the biotechnological process. The selectivity and the activity of the biocatalysts have done many progresses in the last years while the stability of the biocatalyst as well as the bioreactor design were less investigated.

The bioreactor design is indeed dependent on many physical and chemical factors such as the oil/water volumetric ratio, oxygen availability, co-factor regeneration, separation of the water in oil emulsion, diffusive mechanisms between the organic and the aqueous phases and biocatalysts recovery. The only commercial bioreactor design is still based on a CSTR technology which shows several limiting aspects for the industrial application [1]. Cell immobilization technology represents a possibility to design a continuous biodesulfurisation reactor which would be less expansive and more efficient [2]. Oil/water volumetric ratio and finishing steps could be highly improved while the stability of the biocatalyst, co-factor regeneration, by-products inhibition and the diffusive mechanisms have to be still solved

By-products inhibition is particularly important for some strain such as the well-known *Rhodococcus rhodochrous* IGTS8 (ATCC 53968) which exhibits a non-reverse feed-back inhibition by the phenolic compounds as well as by sulfite and sulfate produced during the bioconversion [3]. The minimum concentration of 2-hydroxybiphenyl (2HBP) for the total inhibition of the IGTS8 is 30-40 ppm. The reason of this inhibition is not well-known and it seems to be correlated with a physiological factor. This effect is crucial during the bioconversion due to the accumulation of the by-products into the water phase thus limiting the recovery of the biocatalysts for a continuous process.

The conversion step of the biorefining process is also controlled by diffusive parameters which are limiting of the microbial up-take due to the two phases system. The knowledge of the biological as well as the chemical-physical mechanisms involved in hydrocarbon assimilation by microorganisms is of primary importance for progress in petroleum transformations.

In this context, we have developed a new and versatile approach which allows to extend the scope of biphasic biocatalysis to water insoluble substrates. Our approach is based on the use of supramolecular receptors such as chemically modified cyclodextrines (Fig. 1).

MATERIALS AND METHODS

Chemicals

n-Hexadecane (C16), cyclodextrins and dibenzothiophene (DBT) were obtained from Aldrich Chemicals Co, Sigma Aldrich and Merck, respectively. Other chemicals used were reagent grade and were supplied by Carlo Erba (milan).

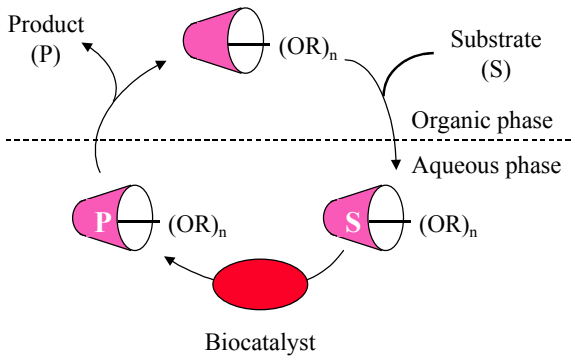

Fig. 1. Inverse phase transfer biocatalysis with supramolecular receptors

Microorganisms and medium

Cells of Rhodococcus rhodochrous IGTS8 (ATCC 53968) were growth in basal salt media supplemented with succinate 1% w/v as carbon source, DBT as sulfur source in dimethylformamide or in C16 and cyclodextrins as supramolecolar receptors. The culture was shaken at 30°C and the growth was monitired with a turbidimeter. To prepare inocula, the culture was grown at 30°C with continuous shaking at 200 rpm in basal salt medium supplemented with succinate 1% and tritiane (0,12 g/l) for 78 h. For all experiments the inoculum size was 10% of the medium volume.

Inhibiting tests

The inhibiting effect of 2-hydroxybiphenyl (2-HBP) on the growth of IGTS8 was assayed in basal salt media supplemented with succinate 1% w7v, dibenzothiophene (0,12 g/l), β-cyclodextrin (10 mM) and 2-HBP at the required concentrations as inhibiting source. The inoculum was prepared in trithiane (0,12 g/l) as previuosly described.

Analysis

DBT residues after the microbial degradation were recovered from the culture medium by liquid/liquid extraction in *n*-hexane. The concentration of DBT were detrmined by spectrophotometric analysis in a Uvikon 860 spectrophotometer (Kontron Instruments), using absorption maxima at 323.8 and 274.7 nm.

RESULTS AND DISCUSSION

Our experiments were performed using a model system constituted by dibenzothiophene and n-hexadecane which represent respectively the organic sulfur compounds and the paraffins of a simplified middle distillate.

The main pathway for the biotransformation of DBT by IGTS8 is characterized by oxidative steps [4], which convert DBT in DBT-sulfoxide and to DBT-sulfone (Fig. 2). The sulfone is transformed to 2-(2′-hydroxypheny l)benzene sulfonate and then to 2-HBP and sulfate by a sulfonic acid hydrolase.

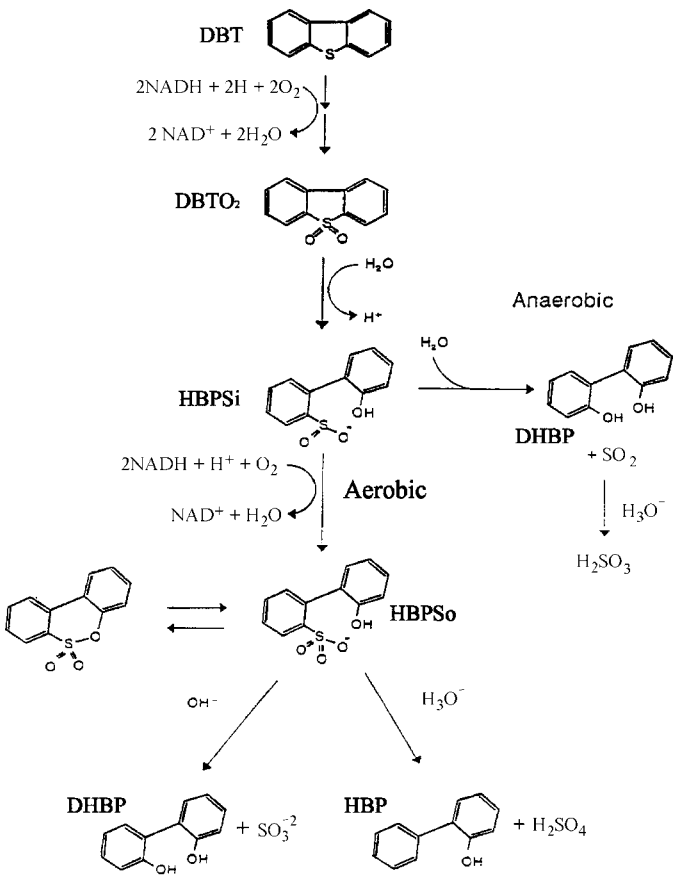

Fig. 2. Scheme of the suggested DBT-metabolizing pathways by R. rhodochrous IGTS8 and the molecular structures of some intermediate metabolites. DBT: dibenzothiophene; DBTO: dibenzothiophene sulfoxide; DBTO2: dibenzothiophene sulfone; HBPSi 2-(2′-hydroxyphenyl)benzene sulfinate; HBPSo 2-(2′-hydroxyphenyl)benzene sulfonate; HBP: 2-hydroxybiphenyl

DBT degradation by Rhodococcus rhodochrous IGTS8 in the presence of β-cyclodextrine

The experiments were performed using both a DBT ($1.9 \cdot 10^{-2}$ mM) and β-cyclodextrine (2.2 mM) concentrations in order to guarantee that the sulfur compound was completely in the adduct formation. The growth rates were significantly higher in the presence of the cyclodextrines while the lag phase was the same (Fig. 3). The DBT conversion rates were higher with respect in the absence of cyclodextrine.

Microbial degradation of hydrocarbons depends on various chemical and physical factors as well as biological. Wodzinski and Coyle (1974) suggested that polycyclic aromatic hydrocarbons (PAHs) can be converted only in the dissolved state [5]. The behavior of our experiments seems to be linked to the stability of the inclusion compounds. In fact the experimental conditions of DBT and CD concentrations were chosen in order to have all the DBT molecules forming a 1:1 complex with the host macromolecules. It is thus reasonable to assume that the cyclodextrine acts as a carrier agent for the transport of the drug through the aqueous medium to the lipophylic absorption site of the cell.

DBT degradation by Rhodococcus rhodochrous IGTS8 in the presence of cyclodextrines and 2-hydroxybiphenyl

The inhibition effect of the 2-hydroxybiphenyl on the DBT bioconversion is well known [6] and for IGTS8 was reported by Setti et al. (1999) [2]. The growth of IGTS8 is completely inhibited at a HBP concentration close to 50 ppm and the growth-limiting concentration was calculated around 30–40 ppm. The experiments carried out in the presence of cyclodextrines have showed that the growth of IGTS8 slightly depended on the increase of the initial concentration of 2-hydroxybiphenyl (2HBP) in the medium (Fig. 4). Similar trends were observed in the DBT conversion rates in which the capacity of the cyclodextrine to

decrease the inhibition effect of the 2HBP was showed. In all experiments DBT resulted completely converted after 250 hours of fermentation.

This phenomena suggests that the cyclodextrine can pick up HBP as guest molecule in solution as well directly at the interphase of the cellular biomembrane thus protecting the microorganisms from the irreversible inhibition effect of this phenols.

DBT degradation by Rhodococcus rhodochrous IGTS8 in biphasic system in the presence of hydroxipropyl-β-cyclodextrine

The choose of the hydroxypropyl-β-cyclodextrine in the experiments with biphasic systems was due to the fact that this molecular receptor is capable to pick-up the DBT from the organic phase constituted by *n*-hexadecane into the aqueous phase where operate the IGTS8. In Fig. 5 the DBT mass transfer in aqueous phase from C16 is reported. β-Cyclodextrine is not capable to favour the DBT mass transfer at any concentration of cyclodextrine in aqueous solution. The concentration of DBT in the aqueous phase increases when the concentration of hydroxypropyl-β-cyclodextrine increases up to a maximum of about 9 ppm of DBT in water for a concentration 0.17M of cyclodextrine. This phenomena is probably linked to the hydroxipropyl that is the functional group used for modify β-cyclodextrine. In fact this group confers a higher hydrophobic characteristic to the β-cyclodextrine that is then capable to work at the interphase between water and C16.

The specific rate for the conversion of DBT in organic phase by IGTS8 is expressed as,

a_s = (concentration in ppm of DBT converted) / hour / gram of dry cell

The specific rate increases when the concentration of DBT in the organic phase increases. This is due to the diffusive effect from the bulk of the organic phase to the interphase with the water phase. In fact when the specific rate is under to a diffusive control, the concentration of the DBT at the interphase can be considered close to zero. In the presence of hydroxypropyl-β-cyclodextrine, the specific rates did not show significantly differences at low concentration of DBT with respect in the system without cyclodextrine while they resulted higher at high concentration of DBT. This behaviour was essentially due to the fact that the mass transfer mechanism at the interphase is strictly dependent to the concentration of the host and guest molecules in the aqueous and organic phase, respectively, in order to form the maximum concentration of supramolecular adduct.

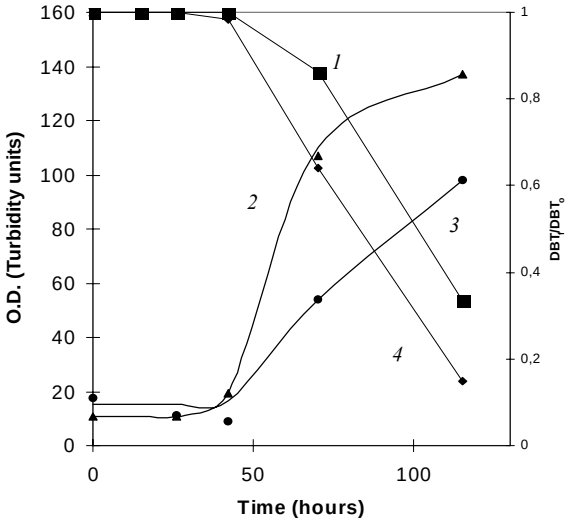

Fig. 3. Growth of IGTS8 on 1% succinate and DBT transformation (120 ppm, the initial concentration) in the presence (s and u, respectively) and in the absence (l and n, respectively) of β-cyclodextrine

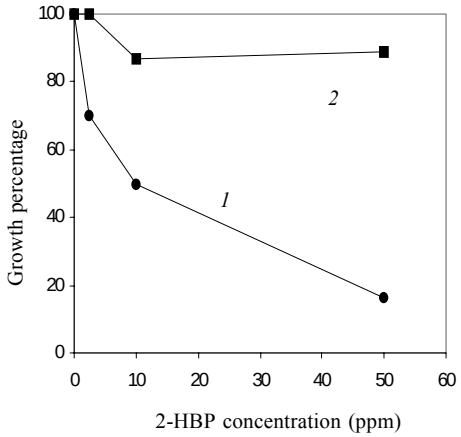

Fig. 4. Growth percentage of IGTS8 on 1% succinate and 120 ppm DBT after 120 h of fermentation in the absence [2] and in the presence of β-cyclodextrine (10 mM) as a function of different initial HBP concentrations in the medium

CONCLUSIONS

In this paper, preliminary results about the use of supramolacular receptors such as modified cyclodextrines in biphasic biocatalysis were reported. The novel approach

Table 1

Specific rate of the DBT converted by IGTS8 expressed as ppm of DBT converted per hour per gram of dry cell as a function of DBT concentration in *n*-hexadecane in absence and in presence of hydroxypropyl-β-cyclodextrine (3.14 mM). The system is constituted by an oil/water volumetric ratio of 1:1 and by an initial biomass concentration of 1g/l

Specific rate (ppm of DBT converted in n-hexadecane / hour / gram of dry cell)		
DBT concentration (ppm)	No cyclodextrine	Hydroxypropyl-β-cyclodextrine
30	1.07	1.07
60	1.1	1.79
120	2.86	4.29
635	21.79	26.10

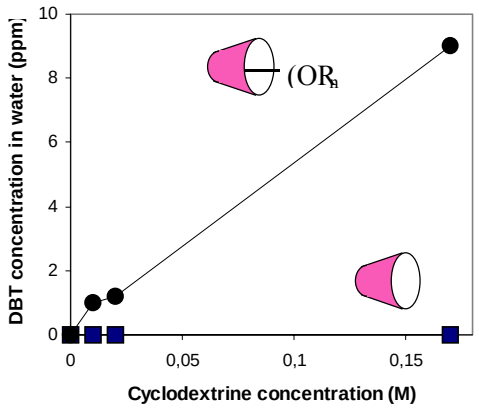

Fig. 5. DBT concentration in aqueous phase (ppm) as a function of the β-cyclodextrine (n) and hydroxypropyl-β-cyclodextrine (l) in the water phase by a mass trasfer of DBT from the organic phase (625 ppm of DBT in n-hexadecane). The system is constituted by an oil/water volumetric ratio of 1:1

can be named as an inverse phase transfer biocatalysis (IPTB) based on supramolecular receptors which selectively pick-up the sulfur aromatic compounds in the organic phase and transfer them into the water phase where the biocatalyst is located. We have observed that the hydroxypropyl-β-cyclodextrine can improve the mass transfer of water insoluble substrates such as dibenzothiophene between the organic phase constituted by n-hexadecane and the aqueous phases. Furthermore, we have evidenced that these macromolecular receptors can decrease and/or eliminate the feed-back inhibition of the biocatalyst due to the by-products accumulation in water phase. Byproducts inhibition is particularly important for some species such as the well-known *Rhodococcus rhodochrous* IGTS8 which exhibits a non-reverse feed-back inhibition by the phenolic compounds produced during the bioconversion. This effect is crucial in the immobilization technology because of the accumulation of the phenols into the hydrogel matrix as a consequence of their high solubility in water. IPTB technology could increase the activity and the stability as well as the selectivity of the biocatalyst in a two phases bioreactor. This technology leads to new perspectives for the cell immobilization because it would overcome the diffusive mechanisms which are the main limiting factors for an industrial application. In particular, it would be possible a cell immobilization by encapsulation technique in hydrogel matrix which highly increases the solvent tolerance of the biocatalyst.

REFERENCES

1. *Monticello D.J.* // NPRA Annual Meeting. 1993.
2. *Setti L., Lanzarini G., Pifferi P.G.* // Immobilized Cells: Basics and Applications (eds. R.H. Wijffels, R.M. Buitelaar, C. Bucke and J. Tramper). Progress in Biotechnology. 1996. Elsevier Science B.V. **11.** P. 777.
3. *Setti L., Farinelli P., Di Martino S., Frassinetti S., Lanzarini G., Pifferi P.G.* // Appl. Microbiol. Biotechnol. 1999. **52.** P. 111.
4. *Oldfield C., Pogrebinsky O., Simmonds J., Olson E.S., Kulpa C.F.* // Microbiology. 1997. **143.** P. 2961.
5. *Wodzinski R.S., Coyle J.E.* // Appl. Microbiol. 1974. **27.** P. 1081.
6. *Nekodzuka S., Toshiaki N., Nakajima-Kambe T., Nobura N., Lu J. Nakahara Y.* // Biocatalysis Biotransformation. 1997. **15.** P. 21.

УДК 577.15

БИОКАТАЛИЗ С ПЕРЕНОСОМ В ОБРАЩЕННОЙ ФАЗЕ ДЛЯ ОБЕССЕРИВАНИЯ НЕФТЯНЫХ ДИСТИЛЛЯТОВ

Л. Сетти, С. Боноли, Е. Бадиали, С. Джулиани

(Департамент Промышленной химии и материалов, Болонья)

При биокаталитическом обессеривании топлив возникает ряд проблем, связанных с ингибированием биокатализаторов продуктами реакции и диффузией между органической и водной фазами. Биокатализ с переносом в обращенной фазе основан на использовании супрамолекулярных переносчиков (модифицированных циклодекстринов), которые селективно захватывают ароматические соединения серы в органической фазе и переносят их в водную фазу, где находится биокатализатор. Он может улучшать массообмен между органической и водной фазами нерастворимых в воде субстратов, а также снижать и/или элиминировать ингибирование биокатализатора из-за накопления продуктов реакции в водной фазе.