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Chromatographic methods applied in the monitoring of biodesulfurization processes – State of the art

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Abstract

Analytical methodologies employed in biodesulfurization processes have been reviewed; attention is primarily focused on the use of analytical techniques in the identification of degradation products and on the monitoring of degradation processes in which microbial sulphur-specific transformations take place. This is the first review of analytical techniques applied to biodesulfurization processes. Methodologies based on gas chromatography (GC) are the most frequently employed, in tandem with various detectors, mainly with the mass spectrometry (MS) detector, and the flame ionization detector (FID). High performance liquid chromatography (HPLC) coupled with ultra violet (UV) detection has also been widely employed. Different sulphurated compounds are used as model in biodesulphurization processes, naphtothiophene (NTH), benzothiophene (BTH), alkilated BTH, dibenzothiophene (DBT), alkilated DBT and their transformation products has been reviewed. DBT is the most frequently employed.

Keywords: Biodesulfurization; Dibenzothiophene; GC–MS; GC–FID; HPLC–UV; Polycyclic aromatic sulfurated hydrocarbons (PASHs); Alkilated dibenzothiophenes

1. Introduction

Crude oil and its distillates contain significant amounts of low-molecular-mass organosulphur compounds such as alkyl- and cycloalkyl thiols, alkyl- and arylthioethers and aromatic heterocycles based on thiophene. This last group of polycyclic aromatic sulfurated hydrocarbons (PASHs) includes thiophene itself, benzothiophene, dibenzothiophene, and their alkylated derivatives. These compounds have been of concern for decades because they constitute a major class of ubiquitous environmental contaminants found in both air and sea areas [1–3]. In order to mitigate the consequences of this contamination, such as acid rain [4,5] and air pollution caused by sulphur dioxide released from the combustion of oils, more and more regulations on sulphur content in petroleum are being established. The current specification in Europe and USA calls for a maximum sulphur content of 50 ppm in gasoline and diesel oil by 2005, and this level will be reduced to below 10 ppm by the year 2010 [6]. The current industrial method used for the removal of sulphur from fuels is hydrodesulfurization (HDS), which requires high temperature and high pressure. HDS is costly energy-intensive for deep desulfurization. Furthemore, HDS is not effective for removing heterocyclic sulphur compounds such as DBT and DBT derivatives [7]. Biodesulfurization has attracted attention owing to its application to the desulfurization of petroleum due to its mild conditions, lower energy consumption and lower emission of CO_2. Thus biodesulfurization (BDS), which operates under room temperature and pressure conditions, is expected to be a complementary as well as promising alternative to HDS. BDS is a process which removes sulphur from fossil fuels using a series of enzyme-catalyzed reactions. It leads to the development of highly efficient reactions and environmentally friendly technologies. Numerous attempts have been made to develop BDS processes. A few strains which can desulfurize DBT and DBT derivatives have been isolated, such as *Rhodococcus erythropolis* IGTS8 [8], *Gordona* sp. CYKD1 [9] *Peanibacillus* sp. A11-2 [10] and *Rodococcus* sp. Strain P 32C1 [11], *Mycobacteium* sp. [12], and *Pseudomonas* sp.[13].

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The desulfurization activity of naturally occurring bacterial cultures is low in comparison to the requirements of a commercial process and genetic manipulation has been used to achieve higher desulfurization rates [14].

Due to the complexity of the system to studying, the biodesulfurization has been conducted preferably in model reactions media in presence or not of water-immiscible organic solvents. Until now, there have been few reports concerning the biodesulfurization of crude oil, hydrodesulfurization-treated diesel oil and gasoline [15–17]. These studies published in 2006 represent a great advance for industrial process implementation.

When a degradation process is performed, the use of analytical techniques to identify and quantify the degradation products is an aspect of interest. Subsequently, analytical techniques which give structural information of intermediates have special interest when a pathway is required. Different analytical techniques have been used with this aim when the degradation of organic compounds under different conditions has been studied. As examples, GC–MS, gas chromatography coupled to atomic emission detector (GC–AED) or liquid chromatography–time of flight–mass spectrometry (LC–ToF–MS) techniques have been used in the determination of transformation products of Imidacloprid (a pesticide), Methyl *tert*-butyl ether (and additive of gasoline) and Bisphenol A (an industrial chemical) in degradation processes such as advanced oxidation processes or sunlight photo-alteration [18–22].

When only monitoring of the process is required more simple analytical techniques can be used, such as, GC–FID, HPLC–UV.

In most cases the concentration of the transformation products in degradation processes is low, so, preconcentration techniques must be employed [18] in order to increase the analytical signal of the transformation products. Besides, sometimes the media in which the degradation is performed is not adequate for analysis under chromatographic techniques; so, a change in the solvent is required before gas or liquid chromatography analysis. All these sample treatments which need to be performed after the biodesulfurization processes will be reviewed in this paper.

When a biodesulfurization process is developed it is necessary to choose a model compound which will be degraded under controlled conditions, the parent compounds used traditionally in biodesulfurization studies will be reviewed in this paper and this revision will focus on the interesting that lies in selecting these compounds in biodesulfurization processes, the analytical techniques employed in the monitoring of the process and the problems concerning the identification of compounds.

Analytical techniques employed in the identification of degradation products in biodesulfurization processes will also be reviewed in the present paper. By using different analytical techniques two pathways for the DBT metabolism are now recognized, namely, the ring-destructive pathway, represented by the Kodama pathway and the sulphur-specific pathway, the know as 4S pathway [23–26]. This review will focus on degradation products of the 4S pathway.

2. Sample treatment

Preconcentration steps after a degradation process are a very important aspect when intermediate products have to be determined, since normally the concentration of unknowns is very low, the extraction procedure is typically optimized for the compounds that will be degradated and for some others which are expected to form if analytical standards are available [17,21,22], then, the application of the method is performed in order to detect all of the transformation products generated in the degradation process. Even when a preconcentration is not necessary, a change of the solvent is normally required, before injection in chromatographic system.

In a biodesulfurization process two phases are typically involved, a watery phase and an organic phase, normally the organic phase is a long chain hydrocarbon such as dodecane. These two phases should be separated and analysed in a different way; the organic phase can be directly analysed by GC or LC or can be extracted by solid phase extraction, and the watery phase can be directly analysed by LC or can be extracted by liquid-liquid extraction if GC analysis is required.

Solid phase extraction sorbents are normally chosen by the nature of their primary interaction or retention mechanisms with the analyte in question. In that case non polar or moderately polar compounds should be extracted from a non polar organic solvent. Sorbents like silica, animopropyl, cyanopropyl are specially indicated to extract compounds with functioanal groups such as hydroxyls, amines and heteroatoms (S, O, N) from non polar matrices.

In fact, the solid phase extraction methods used for the extraction of biodesulfurization compounds (pattern compounds or degradation products) developed by various authors, involve the use of silica packing for the separation and concentration of DBT, 2-HBP (2-hydroxy biphenyl), 2,2′-biphenilol and DBT-sulfone [27], alkylated dibenzothiophenes and its transformation products [24,28].

Different solvents have been used to perform liquid-liquid extraction, ethyl acetate [29–34,26], *n*-hexane [35] methylene chloride [36]. The culture broth is normally acidified before liquid-liquid extraction at pH 2 [26,34].

3. Model compounds used in biodesulfurization processes

3.1. Dibenzothiophene

It is well know that some polycyclic aromatic sulphur heterocycles (PASH) are more recalcitrante than aliphatic sulphur compounds in the catalytic hydrodesulfurization because the sulphur atom is embedded in an aromatic system and furthermore it can be shielded by alkyl groups.

Dibenzothiophene (DBT), is a polycyclic aromatic sulfurated hydrocarbon (see structure in Fig. 1a), which is a representative compound of organic sulphur compounds in fossil fuels. This compound has been the mostly used as a model compound in biodesulfurization processes [29,30,36–39].