

# The effect of spent bleaching earth ageing process on its physicochemical and microbial composition and its potential use as a source of fatty acids and triterpenes

Teresa Krzyśko-Łupicka · Krystyna Cybulska ·  
Andrzej Wiczorek · Ewa Moździerz · Maciej J. Nowak

Received: 13 January 2014 / Accepted: 7 May 2014 / Published online: 31 May 2014  
© The Author(s) 2014. This article is published with open access at Springerlink.com

**Abstract** This study was aimed at investigating the physicochemical and microbiological changes that took place during the ageing process of spent bleaching earth in the presence of autochthonous microorganisms. Research material included fresh spent bleaching earth (SBE<sub>0</sub>) and the same material after 3 years of storage at the constant temperature of 20 °C, without aeration and moistening (SBE<sub>3</sub>). Changes in the chemical composition of analysed waste material were observed during its ageing process point to a spontaneous bioconversion of fat substance towards formation and/or release of free saturated fatty acids C16:0 and C18:0 (14.3 g 100 g<sup>-1</sup> D.M.), triterpenes (8.48 g 100 g<sup>-1</sup> D.M.), cholesterol (3.29 g 100 g<sup>-1</sup> D.M.), small quantities of carbohydrates and esters (0.80 g 100 g<sup>-1</sup> D.M.). This process was accompanied by other changes in

physicochemical parameters of the waste material, such as colour, odour and viscosity, decrease in fat content from 28.27 to 24.6 % and that of soluble forms of metals (Mo, Cu, Fe, Zn, Ni, Cr and Mn), ranging from 25 to 75 %, and an increase in pH, from 3.85 to 4.2. At the same time, changes in the microbial consortium were observed.

**Keywords** Spent bleaching earth (SBE) · Lipolytic microorganisms · Bioconversion · Triterpenes · Saturated fatty acids

## Introduction

Waste generation is an inseparable part of economic activity, and therefore, it is reasonable to minimise both the quantity of generated waste materials as well as their negative environmental impact. In the waste management scheme being valid throughout the EU, there are to be found in the order of priority such sections as recovery of usable components and recycling of components which cannot be directly used Directive 2008/98/EC of the European Parliament and of the Council (EU) of 19 November 2008 (European Parliament 2008). However, waste disposal, particularly through landfilling, is still a disagreeable and undesirable necessity (European Council 1999).

Despite many years of research carried out on the management of spent bleaching earth (SBE), a waste material from fat industry is still serious and unsolved, economic and ecological problem.

The fat industry provides substantial quantities of spent bleaching earth (SBE) each year. For example, it is 40,000 t in Poland, 80,000 t in Japan and 1.0–2.0 million t worldwide (Huang and Chang 2010; Tippkötter et al. 2014). This waste material contains from 25 to 40 % oil and various substances being absorbed during bleaching (Beneke and Lagaly 2002;

Responsible editor: Philippe Garrigues

T. Krzyśko-Łupicka  
Department of Biotechnology and Molecular Biology, Faculty of  
Natural and Technical Sciences, University of Opole, Kard. B.  
Kominka 6a, 45-035 Opole, Poland

K. Cybulska  
Department of Microbiology and Biotechnology of Environment,  
West Pomeranian University of Technology in Szczecin, Szczecin,  
Poland

A. Wiczorek (✉)  
Institute of Chemistry and Fundamentals of Environmental  
Protection, West Pomeranian University of Technology in Szczecin,  
Al. Piastów 42, 71-065 Szczecin, Poland  
e-mail: Andrzej.Wiczorek@zut.edu.pl

E. Moździerz  
Department of Land Reclamation and Environmental Chemistry,  
West Pomeranian University of Technology in Szczecin, Szczecin,  
Poland

M. J. Nowak  
Department of Law and Real Estate Management, West Pomeranian  
University of Technology in Szczecin, Szczecin, Poland

Lara and Park 2003; Ming et al. 2003; Kojima et al. 2004; Park and Ming 2004; Krzyśko-Lupicka 2013). These waste materials are difficult to manage or even to dispose of due to the diversified composition, high quantities of water-insoluble substances, such as fatty acids, macro-elements (sodium, potassium, calcium, magnesium, phosphorus, sulphur and chlorine) and micro-elements (iron, manganese, zinc, copper, iodine, cobalt and selenium), plant pigments and heavy metals (cadmium, mercury, arsenic, lead, chromium and nickel) (Chandra and Sathiavelu 2009; Piotrowska-Cyplik et al. 2013).

Therefore, their decomposition in the environment is inhibited and very slow. Removing process of such waste materials through storage is thus inadvisable due to ecological reasons as these pollutants may penetrate into the ground water and even infiltrate water-bearing horizons (Murray 1989) and may be forbidden by law or significantly limited. Moreover, their open-air storage may cause spontaneous combustion (Kheang et al. 2006).

This type of properties and effects significantly impede waste management and environmental protection, which are warranted in the Polish legal provisions, in particular in the Environmental Protection Act of 24 April 2001 (Polish Parliament 2001) and the Waste Act of 14 December 2012 (Polish Parliament 2012). According to European legislation, SBE is classified as hazardous waste with code 070610\* A (European Commission 2000). Due to its high organic content, which exceeds the 6 % limit of the waste acceptance criteria (WAC) for hazardous waste landfills under the EU Landfill Directive, rough SBE cannot be landfilled (European Council 1999) and must be disposed by other means. These may be both classic and modern processes. Anderson (1996) suggested that SBE could be used as an asphalt additive, replacement for plastic parts in refractories, in cement manufacturing, soil stabilisers and road foundation. The SBE also could be added at 0.5 to 2 % as an ingredient in broiler chicks feed (Blair et al. 1986; Jeon and Son 2007). To ensure safety of the animals and people, such use of SBE shall be governed by European Parliament and Council (2002). The recovered oil from SBE may be used for the production of surfactants, biodegradable polyesters, lubricants, anti-rust products, biofuels (Lara and Park 2004; Park et al. 2008; Huang and Chang 2010; Selvabala et al. 2011; Pourvosoghi et al. 2013) and other chemical compounds (Park and Ming 2004). Tippkötter and co-workers (2014) propose the use of spent bleaching earth as source of glycerol for the anaerobic production of acetone, butanol and ethanol. The possibilities of using the activated SBE as a sorbent of heavy metals are also tested (Seng et al. 2001; Wambu et al. 2009). In the Europe, incineration of SBE remains the main disposal method (Beshara and Cheeseman 2008).

One of the most important and most effective methods of disposal for such waste materials and their removal from the environment is biological decomposition by the microorganisms displaying lipolytic activity. Lipid rich waste is a high-value substrate for microorganisms, being a fully alternative source of carbon and energy.

Under natural conditions, their biodegradation is a spontaneous process taking place with autochthonous microorganisms showing a synergistic action. A factor determining this process is microorganisms' composition and their activity. It particularly depends on the presence of antagonistic microorganisms, properties of pollutants, waste "age", presence of different chemical compounds, temperature, availability of oxygen and nutrients content. Generally, it depends on microbial and physicochemical properties of the environment where this process is taking place. The biomass of microorganisms, in particular the activity of their lipolytic enzymes, should have a significant effect on changes in physicochemical parameters of the environment, inducing a modification of its composition and, in consequence, affecting the rate of biodegradation processes (<http://www.ejpau.media.pl/volume7/issue1/biotechnology/art-01.html>).

Therefore, it is necessary to know the transformations occurring in SBE, particularly fat bioconversion towards development of different chemical compounds and changes in the microbial consortium. In this context, research aiming at investigating the physicochemical and microbiological changes taking place in the process of ageing of spent bleaching earth in the presence of autochthonous microorganisms is reasonable from both economic and legal point of view.

## Materials and methods

Research material obtained from Polish local rapeseed oil refinery was the diatomaceous spent bleaching earth waste (SBE<sub>0</sub>), collected directly from filters after the refining of vegetable oils at fat processing plants, and the same waste being stored for 3 years (SBE<sub>3</sub>) in two plastic screw top barrels, at a constant temperature of 20 °C without aeration and moistening. From each barrel, three samples were collected from a depth of 30 cm. The initial waste (SBE<sub>0</sub>) had the form of a loose, grease mass of yellow colour and characteristic oily odour. The stored SBE (SBE<sub>3</sub>) had the form of a loose grey-brown mass with a characteristic odour of rancid fat. Determinations were made from averaged samples. Each measurement was repeated two times. If the results were significantly different, a third measurement was performed. The obtained results were analysed statistically, using analysis of variance and testing the factors with the Duncan's test with the use of Statistica 5.5 software (StatSoft).

## Research methods

1. Examination of the physicochemical properties of SBE<sub>0</sub> and SBE<sub>3</sub> included the following determinations:

- Exchangeable acidity in a KCL solution using an ELPO N-512 pH meter (Ewing 1981),
- Sulphate sulphur by the nephelometric method, phosphorus by the spectrophotometric method (Ewing 1981; Ostrowska et al. 1991), total nitrogen (Grace Analytical Lab 1994; Ostrowska et al. 1991), chlorides by the Mohr method according to the ISO standard 9297 (1989) and Skoog et al. (1996),
- Metals (Fe, Cu, Mg, Mn, Zn, Cr, Co, Ni, Pb) by the method of atomic absorption spectroscopy (Rauert et al. 1999) using AAS PU-9100X Philips Unicam.

2. Chemical analysis of fatty extracts included:

- Preparation of fatty extracts from the waste materials under analysis,
- Performance of chromatographic and IR spectrophotometric analyses of fatty extracts.

A weighted portion of SBE (5 g) was extracted with n-hexane in a Soxhlet apparatus. Obtained solution has been concentrated in an evaporator until an oily substance was received, and its weight was determined.

The chromatographic analysis gas chromatography–mass spectrometry (GC/MS) (Sparkman et al. 2011) was conducted using a Hewlett Packard 5890 Series II gas chromatograph under the following conditions: HP-5 capillary column, injector temperature 60 °C, temperature gradient 10 °C/min to 280 °C, isothermal heating 20 min [60/10/280–20], detector temperature 280 °C. Samples were dosed by an on-column injection. Identification of fats, their bioconversion products and their quantitative determinations in the extract were made by using *HP Analytical NIST MS Search Program and Spectra Database* software and the results were given in g 100 g<sup>-1</sup> D.M. SBE.

Process of chemical transformations of fatty substances during the fat ageing was observed using the IR spectroscopy (Stuart 2004). The IR spectrophotometric analyses were performed using a Philips Analytical PU 9800 FT-IR Spectrophotometer with a resolution of 2 cm<sup>-1</sup>. Samples, in the form of a film have been applied on a sodium chloride crystal and studied using the wave number range of 4,000–750 cm<sup>-1</sup>.

Microbiological analysis of SBE<sub>0</sub> and SBE<sub>3</sub> included determinations of the quantitative and qualitative microbial composition by the culture method based on the following mediums:

- Czapek's agar with glucose for fungi (Difco Laboratories 1984),
- YPG agar with chloramphenicol 0.1 g/dm<sup>3</sup> for yeast (Difco Laboratories 1984),
- Nutrient LAB-Agar™ (BIOCORP) for bacteria,
- Starch Casein Nitrate Agar (Difco) for actinomycetes,
- Tributyrin Agar (Fluka); neutral Tributyrin (Fluka) for microorganisms displaying lipolytic activity (Difco Laboratories 1984),
- Waksman's agar for microorganisms displaying amylolytic activity (Difco Laboratories 1984),
- Fraser broth for microorganisms displaying proteolytic activity (Difco Laboratories 1984).

After 5 days of incubation at 25 °C, the total number of bacteria and yeast was determined, while that of other microorganism groups was after 10 days (Kelley 2001). The results were given in colony-forming units per gram dry matter of the waste material. The isolated, macroscopically different bacteria colonies were subject to biochemical identification using analytical profile index (API) tests: API 20 NE and ID32GN for Gram-negative rods, API 50 CHB for Gram-positive bacilli, ID32 STAPH for Gram-positive cocci and API 20 Aux and ID32C for yeast. Filamentous fungi were identified based on their morphological features using the diagnostic keys (Pitt and Hocking 1985; Hoog and Guarro 1995; Pitt 1988; Barnett et al. 2000).

## Results and discussion

The initial waste (SBE<sub>0</sub>) had the form of a loose, grease mass of yellow colour with acid reaction (pH 3.85), characteristic oily odour and a fat content of 28.27 % w/w. After 3 years of storage, its physicochemical and organoleptic characteristics underwent distinct alterations. The stored SBE (SBE<sub>3</sub>) had the form of a loose grey-brown mass with pH 4.2, with a characteristic odour of rancid fat and lower fat content (24.6 % w/w). Clearly, lower contents of the extractable forms of metals (Fe, Cu, Zn, Ni, Cr and Mn) were observed in SBE<sub>3</sub>, within a range from 25 to 75 % (Table 1), and twofold lower contents of sulphates and total phosphorus (Table 2). The content of total nitrogen drastically increased, that of chlorides increased almost twofold (Table 2), whereas the lead content increased sixfold when compared to SBE<sub>0</sub> (Table 1). Also, the content of cadmium increased, while cobalt content has not clearly changed.

Changes observed in both, the physical characteristics of the waste material and the content of respective inorganic components, suggest the chemical transformation leading to the conversion of the bond forms of elements during ageing waste. It resulted in an apparent increase or decrease in the content of chemical elements.

**Table 1** The results of chemical analysis of mobile forms of metals in SBE<sub>0</sub> and SBE<sub>3</sub>

	The content of fatty substances (%) mass	The concentration of metals (mg kg <sup>-1</sup> D.M. SBE)									
		Fe	Cu	Zn	Ni	Cr	Pb	Cd	Co	Mn	Hg
SBE <sub>0</sub>	28.27	10,089	6.0	19.5	12.0	6.0	2.0	0.0	2.5	269	0.004
SBE <sub>3</sub>	24.6	6,000	3.0	15.0	3.0	2.5	11.0	0.5	2.0	85.5	<0.004

Results of the instrumental analyses (IR, GC/MS) of fatty extracts from the waste materials being examined also point to the process of chemical transformations during the fat ageing. Based on the IR spectra, it was found that the chemical composition of fatty extracts was diversified (Fig. 1), while the identifiable components of the waste samples under analysis were carboxylic acids, aldehydes, mono-cyclic hydrocarbons, esters and other aliphatic and aromatic compounds containing characteristic atom formations being specified in Table 3.

The chromatographic analysis by the GC/MS method showed, much like the IR spectroscopy, quantitative and qualitative differentiation of components in fatty extracts from SBE<sub>0</sub> and SBE<sub>3</sub> samples (Fig. 2). Its quantitative results are presented in Table 4. The identification of components was made based on the similarity of mass spectra.

In the fatty acid of the initial waste (SBE<sub>0</sub>), mainly oleic acid (C18:1 ω9) was identified, and small quantities of cyclopentaundecanoic acid. On the other hand, in the stored waste (SBE<sub>3</sub>), saturated fatty acids, such as hexadecanoic acid (C16:0), octadecanoic acid (C18:0) and cyclopentaundecanoic acid (C16:0), were found. They could arise in the process of oxidation; the result of which was complete disappearance of oleic acid. But the appearance of cyclic compounds, such as cyclopentaundecanoic acid, triterpenes and cholesterol (Table 4), may be the result of stereomutation with oxidation and formation of cyclic compounds, such as cyclopentaundecanoic acid, triterpenes and cholesterol (Table 4).

The changes observed in the qualitative and quantitative composition of bleaching earth as a consequence of ageing could be both the result of chemical and biochemical transformations of the components being adsorbed on bleaching earth and, at least in part, the result of transformations occurring in the matrix, in this case in spent bleaching earth, leading

to the release of chemical elements and organic compounds being the pollutants of bleached oil, strongly adsorbed during that process. In this way, substances previously immobilized on the matrix become available and detectable by using standard analytical procedures.

The resulting intermediate products of decomposition of fat, such as free fatty acids, aldehydes, triterpenes, as well as changes in the concentration of metal forms and other elements available on the biological transformation can determine the quantitative and qualitative changes in the microbial population. The microbial consortium inhabiting spent bleached earth, the fresh one and that after ageing, was comprised of both bacteria, yeast and fungi not displaying lipolytic activities as well as the microorganisms showing lipolytic activity.

In SBE<sub>0</sub>, no yeasts were found; the reason of which could be a high concentration of oleic acid (Smith and Alford 1966). During the storage of the waste, a new microbial system was developed as a result of succession. The bacterial count decreased hundredfold, as well as that of the microorganisms displaying lipolytic activity decreased tenfold, whereas the number of filamentous fungi increased tenfold (Fig. 3). Microorganisms displaying amylolytic and proteolytic disappeared totally; however, the amount of yeast increased (100 cfu g<sup>-1</sup>) (Fig. 3).

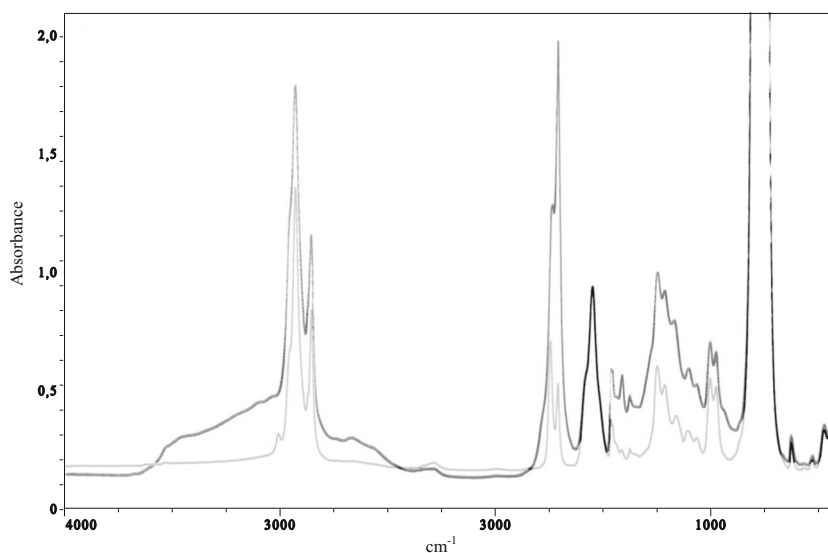
Higher biodiversity of the microorganisms not displaying lipolytic activity was found in SBE<sub>0</sub> 30 species (12 bacteria species and 18 filamentous fungi species), comparing to SBE<sub>3</sub>, where 17 species (nine bacteria species, four yeast species and four filamentous fungi species) were found (Table 5).

Higher diversity was also characteristic for the microorganisms displaying lipolytic activity in SBE<sub>0</sub> (32 species—of which 12 bacteria species) than in SBE<sub>3</sub> (23 species—of which 11 bacteria species) (Table 6).

**Table 2** The inorganic compound content in SBE<sub>0</sub> and SBE<sub>3</sub>

	pH <sub>KCL</sub>	The inorganic compounds (mg kg <sup>-1</sup> D.M. SBE)				Dry mass (%)
		Total nitrogen (N)	Total phosphorus (P)	Chlorides (Cl <sup>-</sup> )	Sulphates (SO <sub>4</sub> <sup>2-</sup> )	
SBE <sub>0</sub>	3.85	0.05	1.64	452	1,650	98.4
SBE <sub>3</sub>	4.2	2,200	0.862	753.4	930	95.9

**Fig. 1** The IR absorption spectra of fatty extracts in the initial waste SBE<sub>0</sub> (black) and the stored waste SBE<sub>3</sub> (grey)



It is characteristic (Table 6) that yeast species displaying lipolytic activity, such as *Sporobolomyces salmonicolor*, *Rhodotorula glutinis*, *Candida colliculosa* (syn. *Torulasporea delbrueckii*) and *Candida* sp., were identified only in SBE<sub>3</sub>.

**Discussion**

The examined spent bleaching earth was a system composed of diatomaceous earth, adsorbed fats, metals and microorganisms, including those being able to grow in the presence of fat or using it in life processes. During ageing, such systems are subject to dynamic changes, both microbiological and chemical ones (Krzyśko-Łupicka 2009). The processes taking place in SBE were slow and extremely complex as a result of high concentration of fat being adsorbed on the carrier, its hampered availability for microorganisms (Wakelin and Foster 1997; Lara and Park 2003; Kojima et al. 2004) and also due to interactions taking place between the metabolites of different populations and the factors activating their growth and activity (Ambachtsheer 2000). The role of mobile metal forms is also

important which, depending on environmental conditions, are adsorbed (immobilised) on diatomaceous earth or released from SBE. A decrease in the content of metals being observed in this study could be a result of their binding by fungi and bacteria during biosorption (Chang and Hong 1994; Mullen et al. 1989; White et al. 1997; Delgado et al. 1998; Yin et al. 1999; Park et al. 2003; Al-Kadeeb 2007), intracellular accumulation or precipitation (formation of chelate complexes) due to which they became non-determinable under analytical conditions.

Observed decrease in sulphate content may be very likely associated with a formation of volatile sulphur compounds, such as hydrogen sulphide, organic sulphides and others, under mild reducing conditions prevailing in the waste material being stored. On the other hand, an increase in the content of total nitrogen is most likely related to the mentioned above reversible sorption–desorption processes of substances containing given chemical elements in the bleaching earth.

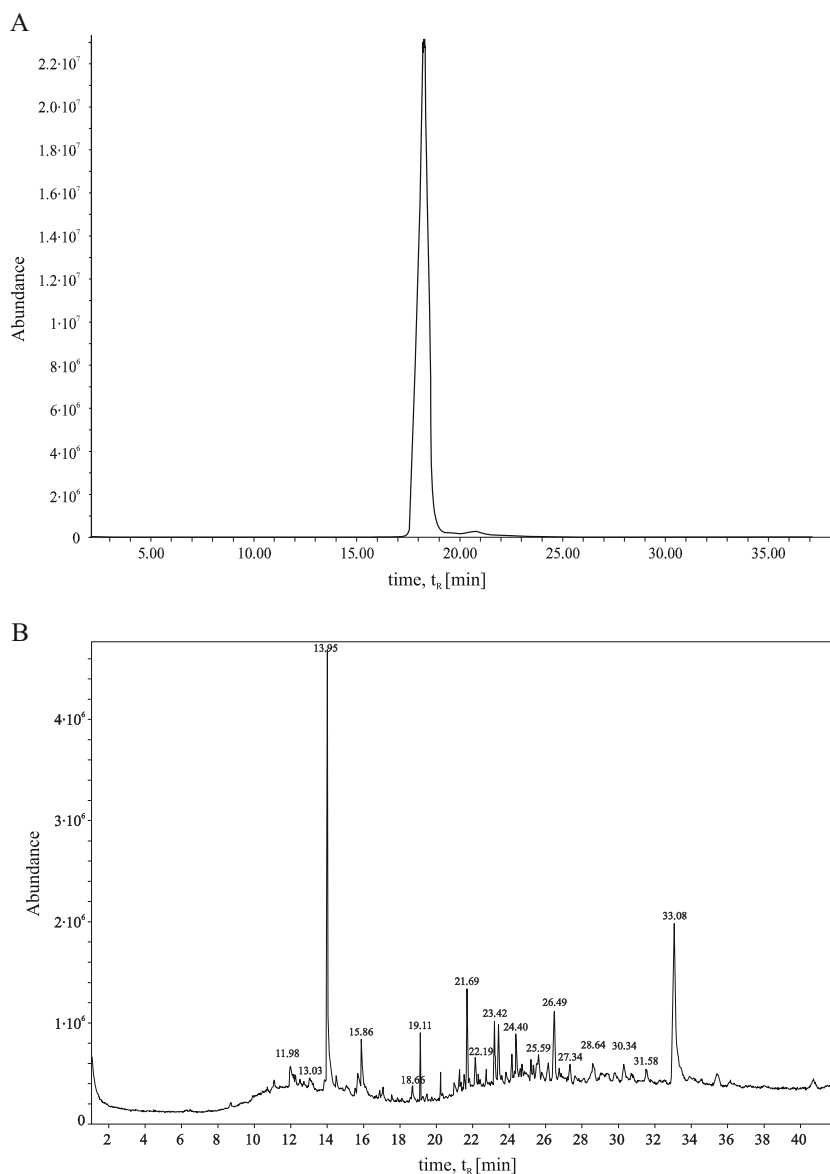
As a result of the waste ageing, an increase in the weight of fatty extracts was observed (from 28.27 g 100 g<sup>-1</sup> D.M. in SBE<sub>0</sub> to 34.08 g 100 g<sup>-1</sup> D.M. in SBE<sub>3</sub>), being an evidence for the course of fat substance bioconversion towards

**Table 3** The IR absorption bands of fatty extracts and corresponding structural elements

Wave number (cm <sup>-1</sup> )	Absorption band	Structural elements
3008	Stretching=CH	-CH=CH-, CR <sub>1</sub> R <sub>2</sub> =CHR <sub>3</sub> , aromatic system
2928, 2856	Stretching C-H (sym. and asym.)	Ar-CH <sub>3</sub> ,-CH <sub>2</sub> - of cycloalkanes
1746, 1711	Stretching C=O	Aldehydes, carboxylic acids, esters
1467	Deformation asym. (def) C-H shear C-H	-CH <sub>3</sub> , -CH <sub>2</sub> -CH <sub>2</sub> -O-, esters
1417	Deformation C-H	CH <sub>3</sub> , CH <sub>2</sub> , C=CH-
1378	Def. sym. C-H	-CH <sub>3</sub>
1252	Stretching C-O	Carboxylic acid esters
978	Def. C-H and C=H	C=C-H
727	Def. out of plane C-H	R <sub>1</sub> CH=CHR <sub>2</sub> (cis), (CH <sub>2</sub> ) <sub>n</sub> n>4 of aliphatic compounds or aromatic system



**Fig. 2** The GC/MS chromatograms of fatty extracts from SBE<sub>0</sub> (**a**) and SBE<sub>3</sub> (**b**)



development of new chemical compounds, better soluble in hexane used during extraction. Considering the high acidity of the examined waste material (pH 3.80–4.20), it is most probable that bioconversion took place with the presence of microflora displaying lipolytic activity and is characterised by low living requirements and high adaptability (Gunstone 1967, 1999; Wakelin and Forster 1997; Edwards 1990; Atlas and Bartha 1998; Brune et al. 2000; Kottler et al. 2001; Das and Dangar 2008; Xiang et al. 2008; Krzyśko-Łupicka 2009; Krzyśko-Łupicka and Robak 2011).

The emergence of new microorganism species in the analysed SBE<sub>3</sub> may be a result of their activation in the presence of newly developed products of fat substance biodegradation (Millet and Lonvaud-Funel 2000; Oliver 2005).

Under these conditions, the products of fat substance degradation and oxidation connected with its transformations

were straight-chain saturated fatty acids (mainly C16:0 and C18:0) and a cyclic fatty acid—cyclopentaundecanoic acid (C16:0), found in substantial quantities in SBE<sub>3</sub>. It can therefore be stated that  $\beta$ -oxidation, hydrogenation, isomerisation and cyclisation processes took place during the storage. On the other hand, oxygen compounds (e.g. aldehydes, ketones) developed probably by hydroperoxide formation and degradation (Gunstone 1967; Park and Ming 2004).

The mechanism resulting in the emergence of other final products, such as triterpenes, in SBE<sub>3</sub> is difficult to explain. However, due to their quantities and biological properties, the waste material being examined may be a new source for obtaining these compounds.

So far, triterpenes, such as lupeol, betulin, demecolcine and cucurbitacin B, have been obtained from many plant species, e.g. from the bark of trees of the family

**Table 4** The quantity and quality composition of free fat in SBE<sub>0</sub> and SBE<sub>3</sub> (g/kg D.M. SBE)

Retention time (min)	Compound	Content of the component in the fatty extract (g 100 g <sup>-1</sup> D. M.)	
		SBE <sub>0</sub>	SBE <sub>3</sub>
Unsaturated fatty acids			
18.3	Oleic acid (C18:1 ω9)	27.14	
Saturated fatty acids			
20.81	Cyclopentaneundecanoic acid (C16:0)	1.13	6.48
13.96	Hexadecanoic acid (C16:0)		5.89
15.86	Octadecanoic acid (C18:0)		1.61
19.11	Cyclopropanenonanoic acid (C12:0)		0.32
	Summary		14.30
Triterpenes			
33.09	Betulin (C <sub>30</sub> H <sub>50</sub> O <sub>2</sub> )		4.23
26.50	Lupeol (C <sub>30</sub> H <sub>50</sub> O)		2.16
27.34	Demecolcine (C <sub>21</sub> H <sub>25</sub> NO <sub>5</sub> )		0.87
13.02	Cucurbitacin B		1.22
	Summary		8.48
Steroid alcohols			
23.43	Cholesterol		3.29
Hydrocarbons			
21.70	1-Docosene		2.11
Ester			
22.18	Tetradecanoic acid, tetradecyl ester		0.80
	Rest compounds		5.10
	Summary	28.27	34.08

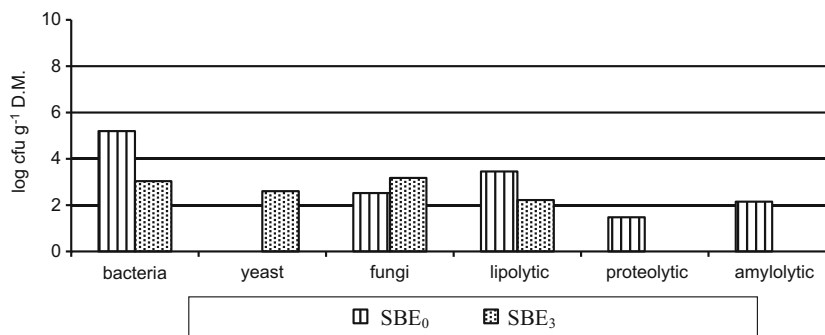
*Betulaceae* in which they occur naturally at the concentration of about 10–30 % (Cole et al. 1991). They exhibit anti-microbial, anti-inflammatory and anti-neoplastic effects and have emulsifying properties. Triterpene compounds (betulinic acid and its derivatives) inhibit proliferation of influenza virus, HSV-1, ECH06 enterovirus or SARS coronavirus (Clinatl et al. 2003; Flekhter et al. 2003; Kanokmedhakul et al. 2003). Until now, the main source of triterpenes has been first of all the liquorice roots and the birch bark (Clinatl et al. 2003) but bioactive substances of that type are also produced by fungi *Sclerotinia citrinum* (Kanokmedhakul et al. 2003).

The emulsifying properties of betulin and its synthetic ester derivatives (tartrate, phthalates, tetrachlorophthalate) are being mainly used in the chemical industry, using them as emulsifiers and stabilisers of the oil/water systems or plasticisers in PCV production.

**Conclusion**

Analysing the n-hexane esters from spent bleaching earth (SBE<sub>0</sub> and SBE<sub>3</sub>), changes were observed in the chemical composition, accompanying the process of its ageing,

**Fig. 3** The effect of waste ageing on quantitative differentiation of microorganisms in SBE<sub>0</sub> and SBE<sub>3</sub> (log cfu g<sup>-1</sup> D.M.) LSD<sub>0,05</sub> bacteria=79307.7, yeast=108.5, fungi=503.3, lipolytic=1982.4, proteolytic=18.0 and amylolytic=82.7



**Table 5** Qualitative composition of the microorganisms not displaying lipolytic activity in the fresh waste (SBE<sub>0</sub>) and after ageing (SBE<sub>3</sub>)

Type of waste	The species composition of microorganisms	
	Bacteria	Fungi
SBE <sub>0</sub>	<i>Burkholderia cepacia</i> , <i>Stenotrophomonas maltophilia</i> , <i>Acinetobacter lwoffii</i> , <i>Aeromonas salmonicida</i> , <i>Bacillus</i> ( <i>B. subtilis</i> , <i>B. cereus</i> , <i>B. firmus</i> ), <i>Staphylococcus</i> ( <i>S. arlettae</i> , <i>S. sciuri</i> ), <i>Rothia mucilaginoso</i> , <i>Xanthobacter</i> sp. <i>Streptomyces</i> sp.	<i>Mucor hiemalis</i> , <i>Rhizopus</i> sp., <i>Mortierella</i> sp., <i>Penicillium</i> ( <i>P. citrinum</i> , <i>P. frequentans</i> , <i>P. notatum</i> ), <i>Aspergillus</i> ( <i>A. nidulans</i> , <i>A. niger</i> ), <i>Acremonium murorum</i> , <i>Trichoderma viride</i> , <i>Scopulariopsis brevicaulis</i> , <i>Cladosporium</i> ( <i>C. cladosporioides</i> , <i>C. fulvum</i> ), <i>Alternaria alternata</i> , <i>Fusarium</i> ( <i>F. solani</i> , <i>F. culmorum</i> , <i>F. sporotrichioides</i> , <i>F. oxysporum</i> )
SBE <sub>3</sub>	<i>Sphingomonas paucimobilis</i> , <i>Bacillus</i> ( <i>B. subtilis</i> , <i>B. cereus</i> , <i>B. firmus</i> , <i>B. lentus</i> ), <i>Staphylococcus</i> ( <i>S. sciuri</i> , <i>S. haemolyticus</i> ), <i>Rothia mucilaginoso</i> , <i>Streptomyces</i> sp.	<i>Rhodotorula glutinis</i> , <i>Sporobolomyces salmonicolor</i> , <i>Pichia</i> sp., <i>Candida</i> sp., <i>Mucor circinelloides</i> , <i>Penicillium citrinum</i> , <i>Aspergillus niger</i> , <i>Cladosporium resinae</i> , <i>Fusarium</i> ( <i>F. aqueductum</i> , <i>F. solani</i> )

pointing to a spontaneous bioconversion of fat substance towards formation and/or release of saturated fatty acids C16:0 and C18:0 (14.3 g 100 g<sup>-1</sup> D.M.), triterpenes (8.48 g 100 g<sup>-1</sup> D.M.), cholesterol (3.29 g 100 g<sup>-1</sup> D.M.), small quantities of carbohydrates and esters (0.80 g 100 g<sup>-1</sup> D.M.). This process was accompanied by physicochemical

**Table 6** Qualitative composition of the microorganisms displaying lipolytic activity in the fresh waste (SBE<sub>0</sub>) and after ageing (SBE<sub>3</sub>)

The presence of lipolytic activity of microorganisms								
Bacteria	Yeast		Fungi		SBE <sub>0</sub>		SBE <sub>3</sub>	
	SBE <sub>0</sub>	SBE <sub>3</sub>	SBE <sub>0</sub>	SBE <sub>3</sub>	SBE <sub>0</sub>	SBE <sub>3</sub>	SBE <sub>0</sub>	SBE <sub>3</sub>
<i>Sphingomonas paucimobilis</i>	+	-	<i>Rhodotorula glutinis</i>	-	+	<i>Mucor hiemalis</i>	+	-
<i>Burkholderia cepacia</i>	+	-	<i>Sporobolomyces salmonicolor</i>	-	+	<i>Mucor circinelloides</i>	-	+
<i>Ochrobactrum anthropi</i>	+	-	<i>Candida colliculosa</i>	-	+	<i>Mortierella</i> sp.	+	-
<i>Pseudomonas alcaligenes</i>	-	+	<i>Candida</i> sp.	-	+	<i>Penicillium citrinum</i>	+	-
<i>Pseudomonas putida</i>	+	-				<i>Penicillium frequentans</i>	+	-
<i>Acinetobacter lwoffii</i>	+	-				<i>Penicillium notatum</i>	+	-
<i>Chryseomonas luteola</i>	-	+				<i>Penicillium albidum</i>	+	-
<i>Bacillus subtilis</i>	+	-				<i>Penicillium</i> sp.	+	-
<i>Bacillus cereus</i>	+	+				<i>Paecilomyces variotii</i>	-	+
<i>Bacillus firmus</i>	+	-				<i>Aspergillus nidulans</i>	+	-
<i>Bacillus lentus</i>	-	+				<i>Aspergillus niger</i>	+	+
<i>Geobacillus stearothermophilus</i>	+	+				<i>Aspergillus terreus</i>	-	+
<i>Bacillus mycoides</i>	+	-				<i>Trichoderma viride</i>	+	-
<i>Bacillus amyloliquefaciens</i>	-	+				<i>Acremonium murorum</i>	+	-
<i>Brevibacillus laterosporus</i>	+	+				<i>Scopulariopsis brevicaulis</i>	+	-
<i>Staphylococcus. warneri</i>	+	-				<i>Cladosporium cladosporioides</i>	+	+
<i>Staphylococcus. haemolyticus</i>	-	+				<i>Cladosporium fulvum</i>	+	-
<i>Staphylococcus. chromogenes</i>	-	+				<i>Cladosporium resinae</i>	-	+
<i>Rothia mucilaginoso</i>	-	+				<i>Alternaria alternata</i>	+	-
<i>Micrococcus luteus</i>	-	+				<i>Fusarium solani</i>	-	+
<i>Kocuria varians</i>	-	+						
<i>Dermacoccus nishinomiyaensis</i>	+	-						
<i>Streptomyces</i> sp.	+	-						
<i>Flavobacterium</i> sp.	+	-						
<i>Myroides</i> sp.	+	-						
<i>Xanthobacter</i> sp.	+	-						

+ present

- not found



changes in the fat substance (colour, odour and viscosity), a decrease in the fat content (from 28.27 to 24.6 %) and that of soluble forms of metals (Mo, Cu, Fe, Zn, Ni, Cr and Mn), ranging from 25 to 75 %, and an increase in pH (from 3.85 to 4.2). At the same time, changes in the microbial consortium were observed.

The extraction of chemical compounds from the aged spent bleaching earth, occurring in it in substantial quantities, would have been certainly justified from the economic point of view. They might be used in both pharmaceutical and chemical industries and cosmetology (betulin esters with saturated fatty acids) after their previous extraction and separation. In the light of the results being obtained, it appears that spent bleaching earth may be a potential source of chemical compounds of the industrial use.

**Open Access** This article is distributed under the terms of the Creative Commons Attribution License which permits any use, distribution, and reproduction in any medium, provided the original author(s) and the source are credited.

**References**

Al-Kadeeb SA (2007) Effect of lead and copper on the growth of heavy metal resistance fungi isolated from second industrial city in Riyadh, Saudi Arabia. *J Appl Sci* 7:1019–1024. doi:10.3923/jas.2007.1019.1024

Ambachtshier R (2000) Bio enhancement of microbial biomass: an appropriate technology. Wisa Biennial Conference, Sun City, South Africa. [http://www.google.com/url?sa=t&ret=j&q=&esrc=s&source=web&cd=1&ved=0CCcQFjAA&url=http%3A%2F%2Fwww.ewisa.co.za%2Fliterature%2Ffiles%2Fambachtsheer.pdf&ei=8pxPU\\_6VJoWM7AbRq4HQDg&usg=AFQjCNGXaG48aQI6kSKVvUQ8YEIRMduryg&bvm=bv.64764171,d.ZGU&cad=rja](http://www.google.com/url?sa=t&ret=j&q=&esrc=s&source=web&cd=1&ved=0CCcQFjAA&url=http%3A%2F%2Fwww.ewisa.co.za%2Fliterature%2Ffiles%2Fambachtsheer.pdf&ei=8pxPU_6VJoWM7AbRq4HQDg&usg=AFQjCNGXaG48aQI6kSKVvUQ8YEIRMduryg&bvm=bv.64764171,d.ZGU&cad=rja). Accessed 17 April 2014

Anderson D (1996) A primer on oils processing technology en. In: Hui Y (ed) *Bailey’s industrial oil and fat products*, 4th edn. Wiley, New York, pp 27–31

Atlas RM, Bartha R (1998) *Microbial ecology, fundamentals and applications*. Benjamin/Cummings Publishing Company Inc, USA

Barnett JA, Payne RW, Yarrow D (2000) *Yeast characteristics and identification*, 3rd edn. Cambridge University Press, Cambridge

Beneke K, Lagaly G (2002) From Fuller’s earth to bleaching earth: a historical note. *Newsletter* 5:57–78

Beshara A, Cheeseman CR (2008) Stabilization and solidification of spent bleaching earth using cement kiln dust, International Waste Working Group (IWWG), Crete 2008 Proceedings. [http://www.google.com/url?sa=t&ret=j&q=&esrc=s&source=web&cd=1&cad=rja&uact=8&ved=0CCcQFjAA&url=http%3A%2F%2Fwww.srcosmos.gr%2Fsrcosmos%2Fshowpub.aspx%3Faa%3D13099&ei=dT1MU5\\_JA-fy7AaSpoGYAQ&usg=AFQjCNFghNCnQhghLzHfFjm0eauvftVdA&bvm=bv.64542518,d.ZGU](http://www.google.com/url?sa=t&ret=j&q=&esrc=s&source=web&cd=1&cad=rja&uact=8&ved=0CCcQFjAA&url=http%3A%2F%2Fwww.srcosmos.gr%2Fsrcosmos%2Fshowpub.aspx%3Faa%3D13099&ei=dT1MU5_JA-fy7AaSpoGYAQ&usg=AFQjCNFghNCnQhghLzHfFjm0eauvftVdA&bvm=bv.64542518,d.ZGU). Accessed 15 April 2014

Blair R, Gagnon J, Salmon RE, Pickard HD (1986) Evaluation of spent bleaching clay as a feed supplement in broiler diets. *Poult Sci* 65(12):2281–2291

Brune A, Frenzel P, Cypionka H (2000) Life at the oxic-anoxic interface: microbial activities and adaptations. *FEMS Microbiol Rev* 24:691–710. doi:10.1111/j.1574-6976.2000.tb00567.x

Chandra M, Sathiavelu S (2009) Waste management in the oil industry in the Mediterranean region by composting. *Clean Techn Environ Policy* 11:293–298. doi:10.1007/s10098-008-0196-x

Chang J, Hong J (1994) Bio-sorption of mercury by the inactivated cells of *Pseudomonas aeruginosa* PU 21 (Rip 64). *Biotech Bioeng* 44: 999–1006. doi:10.1002/bit.260440817

Clinatl J, Morgenstem B, Bauer G, Chandra P, Robenau H, Doerr HW (2003) Glycyrrhizin, an active component of liquorice roots and replication of SARS-associated coronavirus. *Lancet* 361:2045–2046. doi:10.1016/S0140-6736(03)13615-X

Cole B, Bentley M, Hua Y (1991) Triterpenoid extractives in the outer bark of *Betula lenta* (black birch). *Holzforschung* 45(4):265–268

Das J, Dangar TK (2008) Microbial population dynamics, especially stress tolerant *Bacillus thuringiensis*, in partially anaerobic rice field soils during post-harvest period of the Himalayan, island, brackish water and coastal habitats of India. *World J Microbiol Biotechnol* 24:1403–1410. doi:10.1007/s11274-007-9620-3

Delgado A, Anselmo AM, Novais JM (1998) Heavy metal: bio-sorption by dried powdered mycelium of *Fusarium flocciferum*. *Water Environ Res* 70:370–375. doi:10.2175/106143098X125019

Difco Laboratories (1984) *Difco manual. Dehydrated culture media and reagents for microbiology*, 10th edn. Difco Laboratories, Detroit

Edwards C (1990) *Microbiology of extreme environments*. McGraw - Hill Publ Company, New York

European Commission (2000) Commission Decision of 3 May 2000 (with amendments) establishing a list of wastes pursuant to Article 1(a) of Council Directive 75/442/EEC on waste and Council Decision 94/904/EC establishing a list of hazardous waste pursuant to Article 1(4) of Council Directive 91/689/EEC on hazardous waste. *OJ L* 226/3, 6 September 2000

European Council (1999) Council Directive 1999/31/EC on the landfill of waste, *OJ L* 182/1, 16 July 1999

European Parliament (2008) Directive 2008/98/EC of the European Parliament and of the Council of 19 November 2009. *OJ EU L* 312/3, 22 November 2008

European Parliament and Council (2002) Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed - Council statement. *OJ EU L* 140/22, 30 Mai 2002

Ewing GW (1981) *Instrumental methods of chemical analysis*, 4th edn. Mac Grow-Hill Book Company, Inc, New York

Flekhter OB, Boreko EJ, Nigmatullina LR, Pavlova NJ, Galin FZ, Tolstikov GA (2003) Synthesis and antiviral activity of hydrazides and substituted benzal benzalhydrazides of betulinic acid and its derivatives. *Bioorg Khim* 29:326–332

Grace Analytical Lab (1994) Standard operating procedure for total Kjeldahl nitrogen (Lachat Metod). 536 South Clark Street, 10th Floor Chicago, IL 60605

Gunstone FD (1967) *An introduction to the chemistry and biochemistry of fatty acids and their glycerides*. Chapman and Hall LTD. 11 New Letter Lane EC4

Gunstone FD (1999) Enzymes as biocatalysts in the modification of natural lipids. *J Sci Food Agric* 79(12):1535–1549. doi:10.1002/(SICI)1097-0010(199909)79:12<1535::AID-JSFA430>3.0.CO;2-7

Hoog GS, Guarro J (1995) *Atlas of clinical fungi*. Centraalbureau voor Schimmelcultures Bearn and Delft, The Niderlands, Universitat Rovira i Virgili

Huang YP, Chang JI (2010) Biodiesel production from residual oils recovered from spent bleaching earth. *Renew Energy* 35(1):269–274. doi:10.1016/j.renene.2009.07.014

ISO 9297 (1989) *Water quality—determination of chloride—silver nitrate titration with chromate indicator (Mohr’s method)*, ISO 9297

- Jeon HY, Son JH (2007) Studies on evaluation of spent bleaching clay as a feed ingredient in broiler chicks Korean. *J Poult Sci* 34(2):99–104. doi:10.5536/KJPS.2007.34.2.099
- Kanokmedhakul S, Kanokmedhakul K, Prajuabsuk T, Soyong K, Kongsaree P, Suksamran A (2003) A bioactive triterpenoid and vulpinic acid derivatives from the mushroom *Scleroderma citrinum*. *Planta Med* 69:568–571. doi:10.1055/s-2003-40639
- Kelley SG (2001) Basic microbiology techniques. Star Publishing Company, Belmont
- Kheang LS, Foon CS, May CY, Ngan MA (2006) A study of residual oils recovered from spent bleaching earth: their characteristics and applications. *Am J Appl Sci* 3(10):2063–2067
- Kojima S, Du D, Sato M, Park EY (2004) Efficient production of fatty acid methyl ester from waste activated bleaching earth using diesel oil as organic solvent. *J Biosci Bioeng* 98(6):420–424. doi:10.1016/S1389-1723(05)00306-3
- Kottler BDK, White JC, Kelsey JW (2001) Influence of soil moisture on the sequestration of organic compounds in soil. *Chemosphere* 42: 893–898. doi:10.1016/S0045-6535(00)00194-6
- Krzyśko-Lupicka T (2009) Dynamics of microbiological changes and fat transformation in oily bleaching earth according to its modification. *Studies and Monographs*. University of Opole, Publishing University of Opole, Opole (in Polish)
- Krzyśko-Lupicka T (2013) Spent bleaching earth—obtaining and directions of utilization. *Ecol Chem Eng A* 20(6):543–651. doi:10.2428/eece.2013.20(06)059
- Krzyśko-Lupicka T, Robak M (2011) Yeast *Yarrowia lipolytica* in the biodegradation of fatty substances of the oily bleaching earth. *Acta Sci Pol Biotechnol* 10:29–38 (in Polish)
- Lara AV, Park EY (2003) Lipase-catalysed production of biodiesel fuel from vegetable oils contained in waste activated bleaching earth. *Process Biochem* 38:1077–1082
- Lara AV, Park EY (2004) Potential application of industrial oily wastes on biodiesel fuel production using *Candida cylindracea* lipase in organic solvent system. *Enzym Microb Technol* 34(3–4):270–277. doi:10.1016/j.enzmictec.2003.10.015
- Millet V, Lonvaud-Funel A (2000) The viable but non-culturable state of wine microorganisms during storage. *Lett Appl Microbiol* 30:136–141. doi:10.1046/j.1472-765x.2000.00684.x
- Ming H, Lara Pizarro AV, Park EY (2003) Application of waste activated bleaching earth containing rapeseed oil on riboflavin production in the culture *Ashbya gossypii*. *Biotechnol Prog* 19:410–417. doi:10.1021/bp0257325
- Mullen MD, Wolf DC, Ferris FG, Beveridge TJ, Fleming CA, Bailey GW (1989) Bacterial sorption of heavy metals. *Appl Environ Microbiol* 55:3143–3149
- Murray K (1989) Aquaculture engineering technologies for future. *Inst Chem Eng UK, Symp. Ser. No.111(329)*
- Oliver JD (2005) The viable but non-culturable state in bacteria. *J Microbiol* 43:93–100
- Ostrowska A, Gawliński S, Szczubiałka Z (1991) Methods of analysis and evaluation of properties of soils and plants. *Catalogue*. Institute of Environmental Protection, Warszawa (in Polish)
- Park EY, Ming H (2004) Oxidation of rapeseed oil in waste activated bleaching earth and its effect on riboflavin production in culture of *Ashbya gossypii*. *J Biosci Bioeng* 97(1):59–64. doi:10.1016/S1389-1723(04)70166-8
- Park JK, Lee JW, Jung JY (2003) Cadmium uptake capacity of two strains of *Saccharomyces cerevisiae* cells. *Enzym Microb Technol* 33:371–378
- Park EY, Sato M, Kojima S (2008) Lipase-catalyzed biodiesel production from waste activated bleaching earth as raw material in a pilot plant. *Bioresour Technol* 99(8):3130–3135. doi:10.1016/j.biortech.2007.05.059
- Piotrowska-Cyplik A, Chrzanowski Ł, Cyplik P, Dach J, Olejnik A, Staninska J, Czarny J, Lewicko A, Marecik R, Powierska-Czarny J (2013) Composting of oiled bleaching earth: fatty acids degradation, phytotoxicity and mutagenicity changes. *Int Biodeterior Biodegrad* 78:49–57. doi:10.1016/j.ibiod.2012.12.007
- Pitt JI (1988) A laboratory guide to common *Penicillium* species. CSIRO Division of Food Processing, North Ryde
- Pitt J, Hocking AD (1985) In: Jovanovich HB (ed) *Fungi and food spoilage*. A series of monographs. Academic Press Australia, North Ryde
- Polish Parliament (2001) Environmental Protection Act (in Polish). *JL No. 62. item. 627*
- Polish Parliament (2012) Waste Act (in Polish). *JL No. 0. item. 21*
- Pourvosoghi N, Nikbakht AM, Jafarmadar S (2013) An optimized process for biodiesel production from high FFA spent bleaching earth. *Int J Eng Trans C Aspects* 26(12):1545–1550. doi:10.5829/idosi.ije.2013.26.12c.15
- Rauert G, Lopez-Sanchez JF, Sahuquillo A, Rubio R, Davidson C, Ure A, Quevauviller P (1999) Improvement of the BCR three step sequential extraction of new sediment and soil reference materials. *J Environ Monit* 1:57–61. doi:10.1039/A807854H
- Selvabala V, Selvaraj D, Kalimuthu J, Periyaraman P, Subramanian S (2011) Two-step biodiesel production from *Calophyllum inophyllum* oil: optimization of modified b-zeolite catalyzed pretreatment. *Bioresour Technol* 102:1066–1072. doi:10.1016/j.biortech.2010.08.052
- Seng CE, Lee CG, Liew KY (2001) Adsorption of chromium(VI) and nickel(II) ions on acid- and heat-activated deoiled spent bleaching clay. *J Am Oil Chem Soc* 78:831–835. doi:10.1007/s11746-001-0351-7
- Skoog DA, West DM, Holler FJ (1996) *Fundamentals of analytical chemistry*. International Edition, 7th edn. Saunders College Publishing, Philadelphia
- Smith JI, Alford JA (1966) Inhibition of microbial lipases by fatty acids. *Appl Microbiol* 14:694–705
- Sparkman OD, Penton ZE, Kitson FG (2011) *Gas chromatography and mass spectrometry*. A practical guide. Elsevier, Oxford
- Stuart BH (2004) *Infrared spectroscopy: fundamentals and applications*. Wiley, Chichester
- Tippkötter N, Wollny S, Suck K, Sohling U, Ruf F, Ulber R (2014) Recycling of spent oil bleaching earth as source of glycerol for the anaerobic production of acetone, butanol, and ethanol with *Clostridium diolis* and lipolytic *Clostridium lundense*. *Eng Life Sci* 00:1–8. doi:10.1002/elsc.201300113
- Wakelin NG, Forster CF (1997) An investigation into microbial removal of fats, oil and greases. *Bioresour Technol* 59:37–43. doi:10.1016/S0960-8524(96)00134-4
- Wambu EW, Muthakia GK, Shiundu PM, wa Thiongo KJ (2009) Kinetics of copper desorption from regenerated spent bleaching Earth. *Am Eurasian J Sustain Agric* 4:317–323
- White C, Sayer JA, Gado GM (1997) Microbial solubilization and immobilization of toxic metals: biogeochemical processes for treatment of contamination. *FEMS Microbiol Rev* 20:503–516. doi:10.1016/S0168-6445(97)00029-6
- Xiang SR, Doyle A, Holden PA, Schimel JP (2008) Drying and rewetting effects on C and N mineralization and microbial activity in surface and subsurface California grassland soils. *Soil Biol Biochem* 40: 2281–2289. doi:10.1016/j.soilbio.2008.05.004
- Yin P, Yum O, Jin B, Ling Z (1999) Biosorption removal of cadmium from aqueous solution by using pretreated fungal Biomass culture d from starch wastewater. *Water Res* 33(8):1960–1963. doi:10.1016/S0043-1354(98)00400-X