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Review

Overview of bioremediation with technology assessment and emphasis on fungal bioremediation of oil contaminated soils

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ABSTRACT

Environmental contamination is a problem that requires sustainable solutions. Bioremediation technologies have been developed in the last decades and are increasingly used to mitigate environmental accidents and systematic contaminations. A review of bioremediation technologies, based on published article and patent documents, is presented for different types of contaminated matrices, bioremediation agents and contaminants. The worldwide database of the European Patent Office was searched using radicals of keyword as well as the International Patent Classification (IPC) to identify patents in our areas of concern. Technological domains, annual filing volume, legal status, assignee countries and development collaborations are presented and examples are discussed. The total number of patents is compared with the total number of articles. A SWOT analysis for bioremediation technologies is presented. The technologies for water (53%), soils (36%), and sludges (11%) are growing yearly at nearly constant rates. The bioremediation agents are predominantly bacteria (57%), enzymes (19%), fungi (13%), algae (6%), plants (4%) and protozoa. The major contaminants are oils (38%), followed by metals (21%), organic waste (21%), polymers (10%), food (5%), cellulose (5%) and biodiesel. Most of the patents are generally originated from China and United States of America. The soils bioremediation technology of oil is centered on bacteria usage (about two thirds of the articles and patents), being fungi a technology with critical mass and high growth potential. A recent trend in oil bioremediation of soils is the combination of bioremediation agents (fungi and bacteria) in the same process, thus making the process more robust to environment changes.

1. Introduction

Nowadays, society is facing the environmental consequences of its crescent industrialization with contaminant compounds being released on a daily basis, causing severe damage to all spheres of life (Gaur et al., 2014). The commonly used physical-chemical methods are not only expensive, but their byproducts are hazardous to the environment (Gaur et al., 2014). Evidence has shown that there are more suitable remediation techniques for the elimination of these environmental contaminants and, among them, bioremediation is a natural treatment which uses organisms or their products to reduce or eliminate the adverse effects of pollutants in the environment (Muñiz-Hernández and Velázquez-Fernández, 2013). The eco-friendly agents of bioremediation can be microorganisms, enzymes or plants (Gaur et al., 2014). Bioremediation is mainly applied to matrices such as soil, sludges and several types of residual waters (Zhang et al., 2017), and it can be applied in-situ or ex-situ in bioreactors. Its attractiveness is in its cost-effectiveness and environmental friendliness, especially if applied in-situ. In-situ attenuation can use strategies such as bioaugmentation through inoculation and/or biostimulation by the addition of microbial growth-promoting formulations (Andreolli et al., 2015). When used for bioremediation of organic contaminants, microorganisms can usually

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convert them into inorganic matter, nutrients and cell biomass (Reineke, 2001). A wide range of microorganisms such as bacteria, fungus, algae and engineered microorganisms have been studied for soil and aquatic environment bioremediation (Alegbeleye et al., 2017).

Oil industry problems from leakages, spills and environmental accidents, involving contaminants such as fossil fuels and their by-products, are frequent (Silva et al., 2014). The need for remediation of these scenarios has become mandatory, and environmental control agencies apply penalties when corporate responsibilities are determined. Polycyclic aromatic hydrocarbons (PAHs) are probably the products of major concern in oil spills due to their hazard characteristics regarding human health, thereby requiring specific remediation processes (Korda et al., 1997; Lamichhane et al., 2017). Several studies have focused on the treatment of water and soils contaminated with PAHs, using solvent extraction, bioremediation, phytoremediation (Nharingo and Moy, 2016; Qin et al., 2009; Suresh and Ravishankar, 2004), chemical oxidation, photocatalytic degradation, electrokinetic remediation, thermal treatment (Gan et al., 2009), enzymatic processes (Eibes et al., 2015) and algae processes (Zeraatkar et al., 2016) focused on specific compounds (Kucharzyk et al., 2017). Combined remediation types have also been used, for instance, chemical and biological treatment (Gan et al., 2009; Kulik et al., 2006).

In mangrove bioremediation of oil spills, especially important for countries such as Brazil, Indonesia and Australia due to their large mangrove areas, the complexity of the metabolic processes needed to degrade PAHs suggests that no particular microorganism completely degrades petroleum, as bioremediation is more efficient when carried out by complex microbial consortia (Santos et al., 2011).

The incentive or obligation for the addition of higher percentages of biodiesel in regular fuel is aimed at increasing the use of renewable energy sources (Meyer et al., 2014), so it's expected that some spill and environment contaminations occur in the near future due to the widespread of its use. Byproducts from biodiesel production can also be an issue of concern. Researchers are studying biodiesel bioremediation, pure and in blends with regular fuel. Biodiesel has a high biodegradability potential due to the presence of readily oxidizable fatty acids and the absence of aromatic hydrocarbons and sulfur (Meyer et al., 2014), making bioremediation of the blends usually enhanced.

Environmental contamination by heavy metal occurs from industries and toxic waste sites, being, among others, lead, cadmium, arsenic, mercury and chromium the major concern due to their toxicity (Gaur et al., 2014; Ojuederie and Babalola, 2017). These metals are non-biodegradable, which leads to their accumulation in the environment and along the food chain, causing many adverse effects (Gaur et al., 2014). Heavy metals are cytotoxic at low concentrations and can lead to cancer in humans (Ojuederie and Babalola, 2017). The physico–chemical methods for heavy metal removal are ion exchange, chemical precipitation, reverse osmosis, bio-piles, bio-slurries, land-filling (Gaur et al., 2014), adsorption and biosorption (Fu and Wang, 2011). Bioremediation of heavy metals is gradually being accepted as the standard practice since it is eco-friendlier and more cost effective, especially when metal concentrations are low (Ayangbenro and Babalola, 2017). It is usually achieved by phytoremediation or microbial bioremediation (Emenike et al., 2018; Ojuederie and Babalola, 2017; Dixit et al., 2015).

The term "organic waste" is sometimes used to refer to hazardous organic compounds and their mixtures, as in the case of the study conducted by Gallizia et al. (2005), though it is also used to refer to biodegradable organic mixtures, being the latter associated to bioremediation as a supplement for biostimulation (Dadrasnia and Agamuthu, 2013; Jamil and William, 2013). Gallizia et al. (2005) studied the bioremediation of mixtures of organic wastes containing benzene, xylene, toluene, tetrachloroethylene, trichloroethylene, dichloroethylenes and vinyl chloride aiming to naturally enhance the decomposition of organic contaminants in harbor sediments, showing the best strategy to be the biostimulation by water oxygenation via air supply.

Regarding bioremediation of food, two areas of research can be found: one related to the food industry wastewater bioremediation (Chiacchierini et al., 2004; Gupta et al., 2016) and the other related to the use of food waste as carbon source or nutrient supplement to the bioremediation of hazardous substances like oil and heavy metals (Joo et al., 2008; Winarso et al., 2016).

Cellulose industry effluents can pose severe damage to the environment if not treated properly, since they contain a mixture of chemicals used in the process and their byproducts, being the organochlorine compounds one of the major concerns. These effluents are mutagenic (Kulshreshtha et al., 2011) and organochlorines have the potential to migrate widely through the ecosystem, ultimately accumulating in the fatty tissues of the organisms (Suntio et al., 1988). Lignin is an aromatic polymer being its derivative compounds responsible for the dark color of effluents, like chlorinated phenols that are degradation products of chlorolignin (Christov and van Driessel, 2003). One of the organisms well known for bioremediation of these effluents is the white-rot fungi (Christov and van Driessel, 2003; van Driessel and Christopher, 2004).

Scientific articles generally serve as metrics for new technologies with a low technological readiness level (DOE, 2011; NASA, 2014). Patent documents may be used as metrics of medium technological readiness level, as most of the readily available technologies are detailed in patents, making them an important metric to broaden the assessment of potential technologies for bioremediation (Horizon, 2020, 2019).

The aim of this paper is the technological assessment of bioremediation technologies using microorganisms in waters, soils and sludges to assess the technological readiness of bioremediation and summarize it using a SWOT matrix. For this, the total number of articles was used as early tendencies of potential future technologies, and patent documents were mapped and analyzed in detail. The subjects were matrix bioremediated (soils, slurries, waters, others), bioremediation agent (fungi, bacteria, enzymes, protozoa, algae, phyto-remediation), and contaminant (oils, metals, biodiesel, cellulose, organic waste, food).

This work was divided into contaminated matrix, bioremediation agent and type of contaminant. Additionally, a section deepens the analysis of the principal contaminant emphasizing the second major matrix and its second more used bioremediation agent, thus choosing fields with enough critical mass of patents and high potential of growing, inferring the availability of potential future technologies, i.e., it emphasizes fungi bioremediation of soils contaminated with oil.

2. Methodology

The patents were obtained using the Questel Orbit database, which was chosen because it contains the European Patent Office worldwide database (EPO, 2019), is relatively comprehensive and all documents have been translated into English, allowing effective searching of patent families.

Patents are territorial and must be filled in the potential countries where fabrication, usage, or commercialization take place. The "patent family" concept is used whenever the same patented technology has several fillings in different countries (EPO, 2017). Throughout the text, the word patent will be used to mean patent families independently of their legal status.

The patents were searched using both International Patent Classification (IPC) when available and radicals of keywords in the claims and independent claims fields in order to obtain plurals and composite words (Table 1). The results obtained with keywords' radicals and IPCs were merged to obtain the maximum number of pertinent documents.

The search of patents by IPC and keyword's radicals allows to retrieve several IPCs, associated with, but different, from those originally

Table 1
Scope of articles and patent search, showing the radicals of keywords and the International Patent Classification (IPC) used.

Subject	Item	Keyword's radicals	IPC
Bioremediation in general	Bioremediation	Bioremed*	
Matrix to be bioremediated	Soils	soil*	B09B
	Waters	water* or wastewater* or (residual and water) or sewage* or aquatic* or marine* or sea* or liquid*	
	Sludges	slurr* or mud* or sludg* or slim*	
Bioremediation agent	Microorganisms	microorganism*	C12R
	Fungi	fung*	C12R001/645
	Bacteria	bacter*	C12R001/01
	Algae	algae* or microalgae*	C12R001/89
	Protozoa	protozoa*	C12R001/90
	Phytoremediation	phyto* or plant* or veget*	
	Enzyme	enzym*	
Contaminant	Oil	petrol* or (crude oil) or hydrocarbon*	
	Food	food*	
	Biodiesel	biodiesel*	
	Polymer	polym* or polyamid*	
	Organic waste	organic and waste*	
	Cellulose	cellulose or paper industr*	
	Metal	metal*	

searched, broadening the technology overview. Technology domains consist of grouping similar patents, according to their own keywords, IPCs and Collaborative Patent Classification, showing relationships and enriching the discussion.

The amounts of articles for each subject were searched on the Web of Science (Web of Science, 2017) and on the "b-on", a National Portuguese Online Knowledge Library database with over 16,750 scientific international publications from 16 publishers, with full text access (b-on, 2017) using keywords applied to the article abstracts.

Both articles and patents were limited to the publication data from 1997 to October 2017. This range of years was chosen due to the interest in recent technologic developments and considering the 20 years of the lifetime of a patent as well as its 18 months sigil period.

The document data set was cleaned to remove duplicated documents and documents that did not specifically cover bioremediation.

The subjects chosen were matrix to be remedied, bioremediation agent, and contaminant (Table 1). A total of 36,655 articles and 2325 patents since 1997 was found associated with bioremediation.

3. Results and discussion

Fig. 1 shows the countries where patents originated from (1A) and where they were filed (1B), the latter being an indicative of potential markets and competitors. The regional filings in the European Patent Office (EPO) and the international fillings trough the World Intellectual Property Organization were excluded as they could lead to erroneous assessment. The technology appropriations are present on all continents and quite spread across countries. China and the United States of America (US) are the countries with more filings. In some countries, patents are being filed by other countries to obtain exclusivity of production and commercialization, such as in Uruguay (3), Peru (9), and Equator (1) in South America, Saudi Arabia in Asia (1), and South Africa in Africa (1).

An initial assessment of patent technological domains showed that the most relevant issues are indigenous bacteria, oil contaminated soil, contaminated soil remediation, petroleum hydrocarbon degradation and hydrocarbon bioremediation, ground water remediation, environment contamination bioremediation, wastewater bioremediation and bioremediation treatment.

Genetic engineering is a new trend, referred to by less than 1% of the patents, and examples are bacterial strain for degradation of sulfonylurea herbicides (Hong et al., 2013), fenoxaprop-p-ethyl hydrolysis esterase gene for hydrolysis of herbicides (Cui et al., 2011), microorganisms for microbial enhanced oil recovery (Kohr, 2010), and carbamate pesticide degrading enzymes (Yan et al., 2015).

Table 2 shows the percentage of patents in accordance to their legal status (granted, pending, lapsed, revoked, alive). Alive patents consist of those granted or pending and may be enforced to control producers and sellers of bioremediation products and processes. Phyto-remediation has the highest percentage of granted patents (47%), pointing to a more stable technology. The pending patents percentage is highest for bacteria and fungi bioremediation (26% and 28%, respectively) which may be due to these patents being more recent or to examination delay of the patent national offices. Enzymes bioremediation has the highest percentage of lapsed patents (30%), pointing to their applicants having no interest in maintaining their filings alive. Algae bioremediation has the highest percentage of expired patents, showing that 25% have more than 20 years from filing. The percentage of revoked patent filings is below 9% for all technologies, pointing to this area of technology not being too prone to litigation. Phyto-remediation has the highest

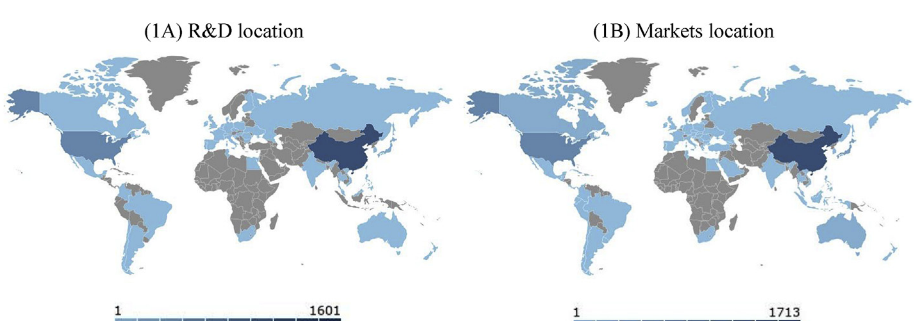

Fig. 1. Countries where the patents of bioremediation technologies are being filed, showing potential competitors (1A) and markets (1B). The gray color countries have no patents. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 2
Percentage of patents legal status (granted, pending, lapsed, revoked) and percentage of alive patents with enforcement potential.

Subject	Granted (%)	Pending (%)	Lapsed (%)	Expired (%)	Revoked (%)	Alive (%) (enforcement potential)
Bioremediation of soils	36	20	28	9	7	56
Bioremediation of sludges	38	18	26	12	6	56
Bioremediation of waters	37	20	26	10	6	57
Bacteria bioremediation	34	26	25	10	5	60
Fungi bioremediation	36	28	23	7	7	64
Enzymes bioremediation	38	20	30	6	7	58
Algae bioremediation	32	21	25	15	8	53
Phyto-remediation	47	23	17	8	6	70

percentage of alive patents (70%), whilst algae bioremediation has the lowest percentage of alive patents (53%). Nevertheless, the alive patents for bioremediation technologies and their uses range from 50% to 70%, showing that it is a technology carefully maintained by their owners, and available for transfer, either by licensing, selling, etc. About 30%–50% of the technology are public domain and may be used without permission from their patent assignees.

3.1. Matrix to be remediated

The matrices studied were soil, waters, and sludge (Fig. 2), focusing on the generally contaminated substrates. The percentages of patents and articles for waters, sludges and soils are almost the same. Waters dominate both articles (Fig. 2A) and patents (Fig. 2B), accounting for about 50% of the documents found. Soils are the second most studied matrix, accounting for 46% of the articles and for 36% of the patents, showing that it is still a relevant issue that is growing up and needs more attention. Sludges are present in 7% of the articles and 11% of the patents.

Fig. 2C shows the accumulated number of patents per priority year for the contaminated matrices. The years 2016 and 2017 are still not complete due to the patent's sigil period of 18 months. The cumulative number of patents on bioremediation is increasing yearly, reflecting the interest in reclaiming contaminated environments. The matrix waters has grown more than the other matrices since 1997, showing that it has more studies and technologies. Soil matrix bioremediation is growing but, due to its relevance for farming and other human activities, still needs more studies, being a promising area of technology development. Sludges have the lowest number of patents, probably because water and soils are considered as higher priorities and because sludge may be included within soils or waters studies.

3.1.1. Waters

Bioremediation of waters is a growing technology. The patent fillings grew at a constant rate (about 90 patents per year) between 2000 and 2004, decreased down to 72 patents in 2007 and grew afterwards (Fig. 2C).

Apparatus and their usage methods for biological treatment of water, wastewater or sewage have technologies filled in more than 10 countries. Examples are bioreactors (Patel, 2017; Perriello, 2001), environmental monitoring and bioprospecting systems (Halden, 2004) and portable decontamination units (Leight et al., 2015).

Biochemistry of microorganisms and their compositions also have technologies filled in more than 10 countries. Examples are nitrite-oxidizing and ammonia-oxidizing bacteria with isolated nucleotide sequences that are freshwater/saltwater tolerant and capable of surviving freeze-drying processes (Hovanec, 2004; Hovanec and Phalen, 2005), lecithin based microemulsions used as nanoreactors or delivery vehicles (Baseeth and Jadhav, 2014), and microbial preparations with increased growth/yield potential and increased survival/recovery rate on a product (Conway et al., 2004).

Most of the patents are from China (850, 582 alive), the US (558, 251 alive), and Japan (127, 50 alive).

The assignees with more patents are the Najing Agricultural University (25, 22 alive), Du Pont de Nemours (22, 17 alive), and the South China University of Technology (11, 9 alive).

The predominant co-working group has as co-assignees Hohai University with the Najing University of Technology and the Nanjing Changjiang Waterway Engineering Bureau which developed controlled-release methods of carbon sources for bioremediation for the China market (Xiaohong et al., 2011). There are several other pairs of coassignees: two from China, one from Canada, and two from the US. Only the Georgia Tech. Research and CSIRO Commonwealth Scientific & Ind. are appropriating their technology with the aim to export bacteria and

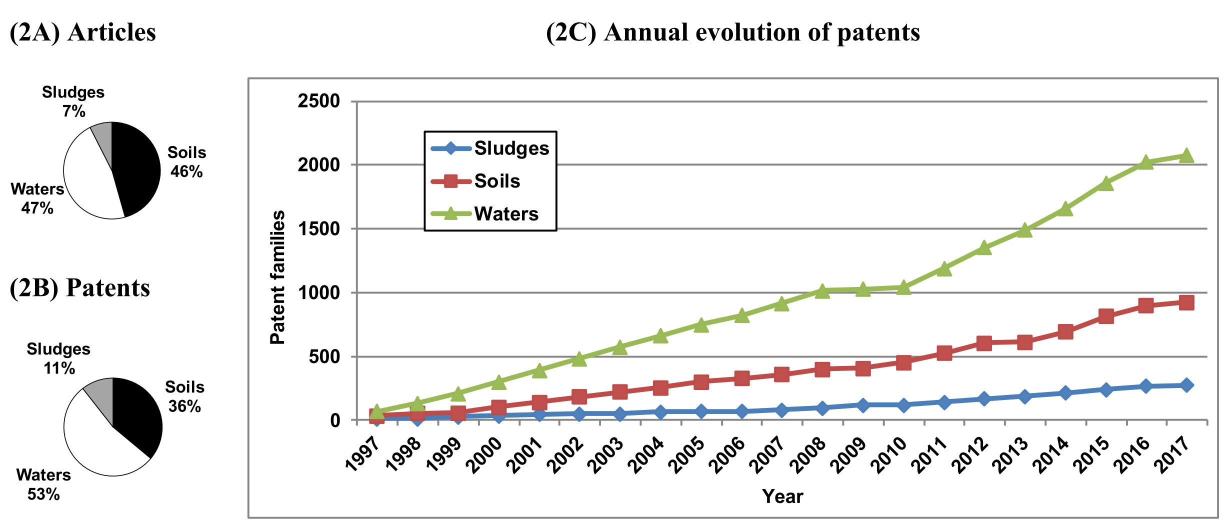

Fig. 2. (2A) Percentage of bioremediation matrix types as reported by articles. (2B) Percentage of bioremediation matrix types as referred to by patents. (2C) Accumulated number of patents per priority year as a function of the matrix being remediated.

enzymes (*Nocardioides* sp. and hydrolase) for the bioremediation of 2,4-dinitroanisole contaminated water bodies (Spain et al., 2016).

3.1.2. Soils

The analysis of the most relevant technological domains of patents that refer to soil bioremediation are contaminated matrix relevance (soil environment, soil remediation, contaminated soil remediation, and associated contaminated ground water), bioremediation agent (fungi, bacteria and microorganisms in general), and contaminants in general and heavy metals in particular.

An example of the microbiologically reclamation operations of contaminated soil with patents filed in more than 6 countries is a surfactant biocatalyst for remediation of recalcitrant organics and heavy metals, developed by the Savannah River companies group, that consists of novel strains of isolated and purified bacteria which have the ability to degrade petroleum hydrocarbons, including a variety of PAHs and to produce biosurfactants, and the combination of biosurfactant-producing ability to degrade PAHs enhances the efficiency with which PAHs may be degraded (Story et al., 2006). Another example is the method and gaseous composition for bioremediation of organic compound contaminations, including halogenated organic compounds and explosives, by injection of a gaseous microbial metabolic inducer and a carrier gas, among other products (Priester et al., 2003).

Most of the patents are from China (323, 219), US (207, 86 alive) and Japan (56, 15 alive).

There are co-assignees with at least three patents co-owned from seven groups. Georgia Tech Research works with the University of Massachusetts to develop the microbial reductive dichlorination of environmental contaminants (Lovley et al., 2010) and with the CSIRO Commonwealth Sci. & Ind. Research Organization to develop bacteria and enzyme exportation technologies (Spain et al., 2016). Nanjing Agricultural University works with the Jiangsu New Ground Biofertilizer Eng. Center to develop microbiological organic products (Qirong et al., 2009a, 2009b), but they do not apply their patents in other countries, thus their technologies are aimed for the Chinese domestic market. The Beth Israel Deaconess Medical Center, the Whitehead Institute for Biomedical Research and the University of Connecticut jointly develop enzymes (Gaxiola et al., 2005; Gaxiola et al., 2009) with applications through the Patent Cooperation Treaty (PCT), aiming to export their technologies.

3.1.3. Sludges

The patents of sludge bioremediation refer mainly to biologic treatments, comprising reclamation operations of contaminated soil, including the biochemistry of microorganisms and their compositions. Examples are biochemical processes for selenium recovery in a bioreactor (Jin et al., 2012) and composite materials and surface-treated calcium carbonate for binding microorganisms capable of degrading hydrocarbon-containing compositions (Di et al., 2013).

Most of the alive patents (granted or pending) were from China (118), followed by the US (32) and Japan (11).

There are two coassignee groups that developed technologies together. Clark Atlanta University and the Microbial Aquatic Treatment Systems jointly developed microbial mats for bioremediation (Bender and Phillips, 1998). China Petroleum & Chemical and Sinopec developed screening methods for denitrifying bacteria (Gao et al., 2009, 2010).

3.2. Bioremediation agents

The bioremediation agents studied were fungi, bacteria, algae, protozoa, phytoremediation, and enzymes. Fig. 3 shows the percentages of each type of bioremediation agent reported in articles (3A) and in patents (3B) as well as the yearly accumulated number of patents (3C). The years 2016 and 2017 are still not complete in terms of patents due to the sigil period of 18 months.

It is possible to observe that, in general, the percentage distributions of bioremediation agents in articles and patents are similar, showing a proportional evolution of technological readiness level from scientific research to patented technologies.

Both articles and patents are focused mainly on bacterial types of remediation (over 55%), probably because this is the more traditional method. Bacteria has an initial steady growth from 1997 to 2001, and, from 2006 onwards, the growth tendency increases.

Enzymes are equally present in patents (19%) an in articles (18%), which was also the case for fungi (14% and 13%, respectively).

Phytoremediation technologies still have low technological readiness level, being more present in articles (7%) than in patents (4%). Algae are more present in patents (6%) than in articles (4%). Protozoa are more referred to in patents (1%), where they are usually associated with other agents.

Although the scientific methodology of mutation and genetic engineering processes has been developed in the seventies, patents referring to bioremediation only started to be filed in 1999, focusing on new plants resistant to contaminated environments and to high salt concentrations (Blumwald et al., 1999; Blum, 1999).

Fig. 3D shows only granted and pending (live) patents for each bioremediation agent versus the five countries with more filings. China (CN) and the US have more patents, followed by Japan (JP), India (IN), and Great Britain (GB). Remediation technologies based on bacteria, fungi, enzymes, and plants have been developed by these five countries. Algae-based technologies were only developed by China and Great Britain, while protozoa-based technologies were developed by all countries except India.

In spite of enzymes as a bioremediation agent having, generally, higher percentage of article and patents (Fig. 3A and B) compared with fungi, it is clear that nowadays fungi are the second most interesting bioremediation agent after bacteria (Fig. 3C and D). So, in terms of microorganisms, fungi seem to be a promising trend to research and develop new technologies. Additionally, fungi are more environmentally friendly when compared with other available remediation methods (Steffen and Tuomela, 2011).

3.2.1. Bacteria

Patent based on bacteria are about 30 per year from 1997 to 2006, increasing up to 105 in 2015. The documents refer to more than seventy types of bacteria, which is to be expected given their specificity to contaminants. The most common are *Pseudomonas* spp., *Rhodococcus* spp. and *Acinetobacter* spp..

Examples are the use of compositions containing *Shewanella* sp., that alter the interface of hydrocarbons and hydrocarbon-coated surfaces, to increase oil recovery, both for bioremediation and for pipeline maintenance (Choban et al., 2010), a mixture of facultative anaerobes capable of metabolizing hydrocarbons under sulfate-reduction conditions (Noland and Elliott, 2004) and butane-utilizing bacteria, which have relatively low trichloroethene toxicity in comparison with conventional methane-utilizing bacteria, and demonstrate an improved ability to degrade hydrocarbon pollutants, such as trichloroethene in the presence of oxygen, by cometabolism or direct metabolism (Perriello, 1999).

Most of the patents originated from China (487, 339 alive), the US (349, 175 alive), Japan (53, 20 alive), Great Britain (31, 12 alive), India (24, 23 alive), and Korea (19, 4 alive).

The assignees with more patents are the Nanjing Agricultural University (26, 23 alive), Du Pont de Nemours (21, 15 alive) and the University of Nankai (12, 4 alive).

There are six pairs of co-assignees, one from Brazil, three from China, and three from the US. Only the US co-assignees are developing exportation technologies: bacteria and enzymes by Georgia Tech. Research and CSIRO (Spain et al., 2016); microbial dechlorination of biphenyls by the Medical University of South Carolina and the University of Maryland (Sowers and May 2002; Sowers et al., 2006); and

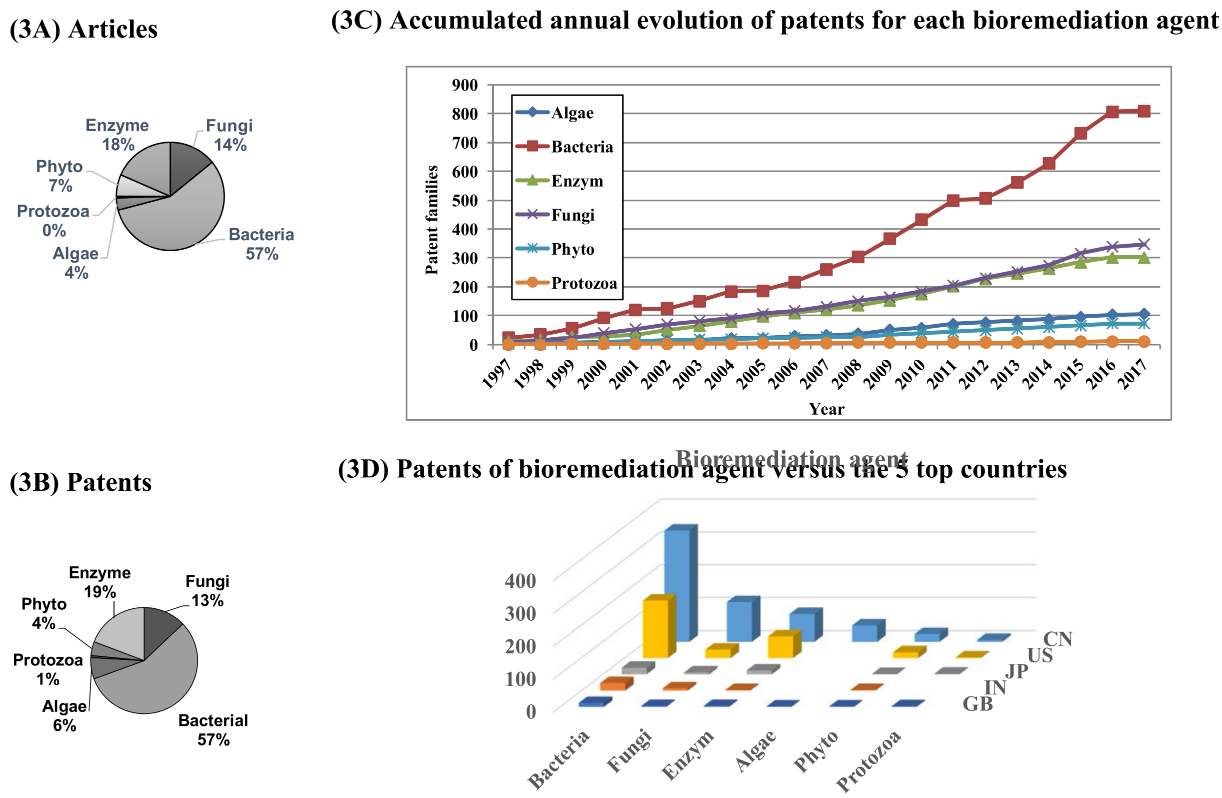

Fig. 3. (3A) Percentages of each type of bioremediation agent reported by articles. (3B) Percentages of each type of bioremediation agent referred to by patents. (3C) Accumulated number of patents per priority year as a function of the bioremediation agent. (3D) Number of granted and pending (live) patents for each bioremediation agent versus the five top countries (CN-China; US-United States of America; JP-Japan; IN-India; GB-Great Britain).

bacteriophages expressing antimicrobial peptides by the Massachusetts Institute of Technology and the University of Boston (Collins et al., 2010).

3.2.2. Fungi

The fungi patents increased yearly, they were homogeneous with quantities similar to those of the enzymes except for recent years, when the former increases (Fig. 3C).

Examples are: the biological treatment of cadmium contaminated water using high-cadmium-adsorption filamentous fungi hair mold *Mucoromyclte* sp. (Chen et al., 2012); preparation of microbial culture and its encapsulation, along with the nutrient requirements inside, with enhanced shelf-life, easy storage and transportation of the formulations at ambient conditions without any substantial loss in viability and in pollutant removing ability (Malik et al., 2015); and remediation of PAHs and heavy metals contaminated soil using white rot fungi spore and fermentation (Cheng et al., 2015).

The documents refer to more than thirty types of fungi. The most common are *Chrysosporium* spp., *Phanerochaete* spp., *Bacillus* spp., *Aspergillus* spp., *Acremonium* spp. and *Penicillium* spp..

Most of the patents originated from China (104, 79 alive), the US (82, 42 alive), Japan (13, 5 alive), and Taiwan (12, 8 alive).

The assignees with more patents are Chromatin (5 live patents), the Institute of Applied Ecology Gas (5 dead patents), Sichuan University (4 live and 1 dead patents), and the US Department of Agriculture and WR Grace, both with 5 dead patents each. Linghua Biotechnology has 4 live patents.

The University of Chicago and Chromatin are co-assignees of two patents that refer to fungi to increase revenues from crops and were appropriated through the PCT, focusing on technology exportation (Mach et al., 2003; Zieler et al., 2005).

3.2.3. Enzymes

The annual evolution of enzyme patents tended to increase steadily (Fig. 3C).

Examples are the development of structures that enable a significantly large amount of enzyme to be immobilized on the surface like enzyme/carbon structure forming covalent bonds (Kim, 2011) and enzyme/fiber matrix composite (Kim and Kim, 2011).

The documents refer to more than ten types of enzymes, most commonly proteases, cellulases, lipases, laccases and peroxidases.

Only eleven patents were appropriated through the PCT, and only five were alive.

Most of the patents originated from China (160, 92 alive), the US (88, 57 alive), Japan (20, 6 alive), Great Britain (17, 8 alive), and Korea (11, 5 alive).

The assignees with more patents are the Nanjing Agricultural University (16, 15 alive) and CSIRO (9, 6 alive).

There are several networks of co-assignees from France (1), Germany (1), China (1), and the US (7).

The patent from the Diversa Cooperation uses enzymes, polynucleotides and polypeptides with haloalkane dehalogenase (Short et al., 2005). The CSIRO Commonwealth Scientific & Industrial Research Organization developed methods and enzymes for degrading hydrophobic ester pesticides and toxins, such as insect esterases, and mutants in the bioremediation of hydrophobic ester pesticides and toxin residues, like pyrethroid residues, contaminants of the environment and horticultural commodities (Russell et al., 2003).

3.2.4. Algae

The annual evolution of the algae patents is growing slowly, with still a small number of patents in this area.

The documents refer to more than forty types of algae, most commonly *Chlorella* spp., *Scenedesmus* spp., and *Chlamydomonas* spp..

There are traditional approaches like culturing algae in an attached periphyton bed and running the water to be treated over the periphyton bed, permitting the algae to sequester a pollutant from the water, and harvesting the algae, thereby removing the contaminant (Jensen et al., 2003). Other example of algae patent is the industrial strain of the unicellular green algae *Parachlorella nurekis* that was developed to eradicate cyanobacteria, bacteria, and fungi (Bogdanov, 2011).

Almost all the patents originated from China (60, 30 alive) and the US (55, 27 alive).

The assignees that have more alive patents have only two patents: Auburn University, Leidos, Liyang Jinquan Ecological Sci. & Tech. Park, and Najing Inst. Geography & Limnology CAS.

Although there are not many patents, this technology has six groups of co-assignees, with two being from China and one from the US.

3.2.5. Phyto-remediation

The annual evolution of the phyto-remediation patents is growing slowly and is irregular due to the mall number of patents.

Examples of patents comprehend micro-propagation of plants, comprising the steps of growing an encapsulated mini rhizome or mini stem cutting (Carver and Tiessen, 2013), treating water with ozone prior or after exposing to natural filtration by periphyton (Jensen, 2004), a reporter system capable of giving rise to a directly monitorable phenotypic trait in a plant, in the presence of an outer stimulus of pollutant, and genetically modified plants comprising the reporter system for monitoring soil pollution and with remediation capability (Meier, 2003) and a method for producing plants for bioremediation (Jensen et al., 2003).

Almost all the patents originated from China (30, 24 alive) and the US (29, 18 alive).

Only three patents were appropriated though the PCT. Only one of them was alive, which refers to the use of terpenes for removing petroleum oils from contaminated soils, assigned to the Brazilian ODC AMBIEVO (Loureiro, 2015).

3.2.6. Protozoa

Only 12 patents were found for protozoa-based bioremediation from 1999 to 2016.

Examples are bioremediation of waste compositions under aerobic conditions utilizing highly diverse and multiple microorganisms within a reactor (Gencer et al., 2011), protozoa used as host cells for haloalkane dehalogenases (Short et al., 2005), and methods of attachment of protozoa to microtubes (Kuhn et al., 2009).

All the assignees had only one patent.

Co-assignees analysis showed only one group: The University of Surrey and the Forestry Commission, in Great Britain, which develop charcoal colonized by microbes to form a community of different cooperating microbes in a biofilm (Hutchings et al., 2007).

3.3. Contaminants

Fig. 4 shows the percentages of articles and patents for each type of contaminant. Most of the contaminants reported in articles (Fig. 4A) are oils (45%), followed by metals (32%), polymers (8%), food (7%), organic wastes in general (5%), cellulose (2%), and biodiesel (1%).

In patents, most of the documents also refer to oils (38%), but this percentage is smaller than that of the articles (45%), suggesting that the technologies for oil bioremediation still have potential for growth, thus opening new opportunities for patents on this subject.

Metals have a higher percentage of articles (32%) than of patents (21%), showing also a growth potential for technologies. Organic waste has a higher percentage of patents than of articles, 21% versus 5%, which may be attributed to bioremediation technologies being more developed and, consequently, the scientific research being less active. Polymers and cellulose have slightly higher percentages in patents than in articles, which may not be significant an may be attributed to oil's patents still having low percentage, thus increasing the other contaminants percentages. Food has almost the same percentage of patents (5%) and of articles (4%). Bioremediation of biodiesel contamination is still in its infancy with a relatively small number of articles and patents.

When selecting only the biggest contaminant documents (oil) and the second more intense matrix to be remediated (soils), i.e., bioremediation of oil-contaminated soils (Fig. 5), it was found that about two thirds of the articles (64%) and two thirds of the patents (70%) referred to bacteria as the biological agent. Fungi bioremediation was second both in articles (17%) and in patents (21%), showing that this remediation agent already has a high potential for further technological development associated with critical mass to support its growth, being worthwhile to deepen its technological assessment. Thus, the next section emphasizes fungal bioremediation of soils with oil spills.

3.4. Fungal soil bioremediation of oil contamination

This section deepens the technological assessment of oil bioremediation, the most referred to contaminant, within the matrix and with the bioremediation agent that have the highest potential of development of future technologies. Soils and fungi were chosen, due to their opportunities for further development, as they are second both in articles and patents.

As examples of the patents that use fungi for soil bioremediation after oil spills those that referred to *Aspergillus* or *Penicillium* were selected due to their wide availability. The patents are from the US, Great Britain, China, the European Community, and Brazil.

The patent "Delivery systems for mycotechnologies, mycofiltration, and mycoremediation", from Cisco Technology, uses fungal spore mass or hyphal fragments in landscaping cloths, fiber substrates, paper products, hydroseeders, and agricultural equipment. The fungi may include saprophytic fungi, gourmet and medicinal mushrooms, mycorrhizal fungi, entomopathogenic fungi, parasitic fungi, and fungi imperfecti. The fungi act as keystone species for the bioremediation of toxic wastes and polluted sites (Stamets, 2002).

The invention "Process for preparing solid fungus to degrade petroleum", from the Institute of Applied Ecology and the Chinese

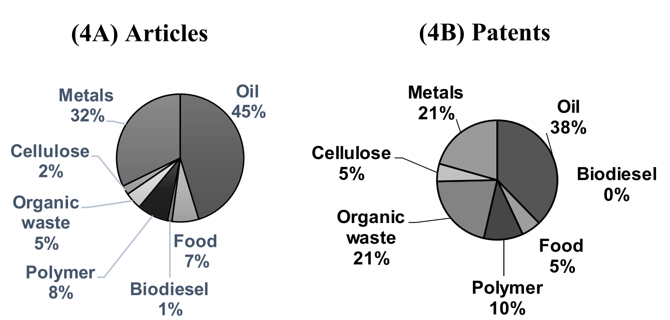

Fig. 4. Percentages of the types of contamination to be bioremediated: (4A) reported by articles; (4B) referred to by patents.

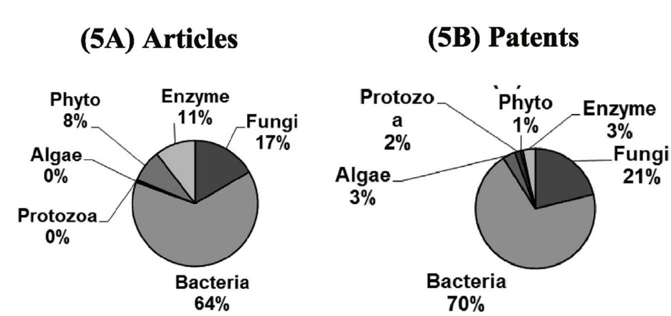

Fig. 5. Percentages of types of bioremediation agents used in soils contaminated with oil as reported by articles (5A) and referred to by patents (5B).

Academy of Sciences, refers to a solid microbe preparation to degrade petroleum. It is prepared from *Mucor*, *Penicillium*, *Aspergillus*, *Trichoderma* and white-rot fungi through slant culture, shake-flask culture, or solid culture, with proportional mixing (Li et al., 2001).

The invention “Process of biodegradable attainment of products for application in the remediation of ground, contaminated waters, and environments and method of functioning”, from the Brazilian Federal University of Bahia, refers to an in-situ process to obtain biodegradable products and to bioremediate contaminated soils and mangroves of non-autochthonous chemical substances or chemical substances such as spilled oil, residual oil, fuel oils, polishes, fats, aromatic hydrocarbons, naphthalene, and polycyclic hydrocarbons. The mix uses *Aspergillus* sp. and byproducts of biodiesel (crude glycerin and castor cake) and acts on bioremediation of mangrove sediment containing sand and saline water (Quintella and Goncalves, 2010).

The invention “In-situ biological repairing method for biomass intensified petroleum contaminate soil”, jointly developed by China Petroleum & Chemical and Tsinghua University, uses a complex of bacteria and fungi to promote the conversion of biomass in polluted soil. The intermediate product serves as a preferential carbon source of the bacteria, promoting biological accumulation. During growth, the fungi act as a surface-active agent, increasing the biological action and accelerating the biotransformation of pollutants. Humus of the transformation product can improve the granular structure of the soil and increase its fertility (Liu et al., 2007a).

The invention from China Petroleum & Chemical “Biological in-situ renovation method for biomass strengthen mix contaminated soil of stone oil-salt" adds a biomass degradation agent, consisting of bacteria (*Pseudomonas, Rhodococcus, Micrococcus, Enterobacter*, *Corynebacterium*, *Bacillus subtilis*, and at least one retinoid) and fungi (*P. chrysosporium*). Then, the biomass degradation agent is plowed and landfilled below the plough horizon. Water logging and salt washing are used to reduce soil salt content and the bacteria and fungi agents are then added. The biomass is used to interdict salt capillary up-rise. The carbohydrates generated by the biomass are used as a good-quality carbon source for the bacteria, accelerating their growth. Lignin generated by the biological degradation can absorb petroleum hydrocarbon pollutants (Liu et al., 2007b).

The invention “Microorganism product for repairing soil polluted by petroleum and products produced thereby and repair method”, from the Chinese Tianjin Ruifengyuan Bioremediction, consists of 60–70% of fungi and 30–40% of bacteria. The fungi are *Aureobasidium Pullulans* (deBary) Arnaud, *Penicillium Freguentans* Westling, *Aspergillus versicolor*, *Penicillium Chrysogenum* Thom, *Fusarium* Lk., *Cephaesosp Orium* Oud., *Tichoderma viride* Pers. Ex Fr., and *Aspergillus niger* Nan. The bacteria are *Acetobacter*, *Bacillus* (*Bacillus*), *Alcaligenes*, *Flavobacterium*, *Arthrobacter*, and *Micrococcus*. They are mixed, diluted with surfactant, and sprayed periodically. Water is supplemented and the plowing and oxygen solarization of the soil are carried out until the required indices are achieved. This invention fixes degrading microbe mixture on wheat bran, solving the problem of long-term storage of the microorganisms, and the activation can be started with warm water. The method can be applied in-situ or ex-situ (Xie et al., 2008).

The invention “Electrically-assistant microbial remediation method of oil polluted soil”, from the Chinese Northeast Forestry University, discloses an ex-situ procedure which comprises the following steps: (1) addition of the oil-contaminated soil; (2) addition of a nutrient solution containing nitrogen and phosphorous; (3) addition of a microorganism mix consisting of bacteria and fungi that may include *Fusarium subglutinans*, *Pseudallescheria boydii*, *Eupenicillium crustaceum*, *lsaria farinosa*, *Aspergillus terreus*, and *Aspergillus protuberus*; (4) addition of organic additives such as surfactants; (5) addition of a wire mesh consisting of a resistance wire heating plate, a parallel temperature controller, and an external power plug; (6) placing the mixture into a reaction tank with the plate on the bottom and turning on the power (Jia et al., 2013).

The patent granted in the US “Composition and methods of use”, from Janet Angel, refers to the use of microbes, enzymes, emulsifiers, and nutrients. The microbes may be bacteria, fungi, algae, or any combination of them. The composition can remove, metabolize, or degrade a hydrocarbon in a contaminated area (Angel, 2014).

The patent “Biocatalytic composition for treatment of substrates”, from Elio Fabio Bortoli, filed in the European Community, US, and Mexico, refers to a biocatalytic composition to be used in the agricultural, zootechnical, and environmental recovery fields to transform substrates, comprising: a component with coenzymatic activity, including vitamin A, vitamin D3, vitamin E, propyl gallate, raw fats, raw proteins; a component with enzymatic activity comprising *Bacillus licheniformis*, *Bacillus subtilis*, *Bacillus thuringiensis*, *Bacillus* spp., *Aspergillus oryzae*, *Aspergillus niger*, *Lactobacillus bifidus*, *Lactobacillus acidophilus*, amylase, protease, lipase, cellulase, gumase; and a component, including substances regulating pH, comprising humic acids, fulvic acids, crenic acids, apocrenic acids, vitamin A, vitamin D3, vitamin PP, and *Arthrospira maxima*, among others (Bortoli, 2015).

The invention “Composite inoculant for deep oil-contaminated soil bioremediation processes”, filed by the Chinese Xi An Huanuo Environment Protection, refers to a composite inoculant for the bioremediation of deep oil-contaminated soil, comprising at least *Aspergillus niger*, *Aspergillus versicolor*, *Penicillium chrysogenum*, *Trichoderma viride*, *Aureobasidium pullulans*, and *Bacillus acidophaeus*. Comparison and screening are carried out to obtain a group of mixed biomass composed of bacteria and fungi, and their proportion is optimized to obtain the composite inoculants. The degradation of petroleum hydrocarbon in soil can reach 90% or more (Zhang, 2016).

As a general trend, it was found that several patents of oil bioremediation of soils use a combination of bioremediation agents, namely fungi and bacteria.

3.5. Bioremediation swot analysis

Strengths, Weaknesses, Opportunities and Threats (SWOT) analysis are usually used to help positioning the technology and serve as a source of information for policies and strategic decisions by the academic researchers, companies and government (Nikolaou and Evangelinos, 2010; Rachid and El Fadel, 2013). Based on the articles and patents, and the authors' critical evaluation, a SWOT analysis of bioremediation technology at international level was performed and a matrix was constructed (Table 3).

4. Conclusions

The bioremediation technologies tendencies with low technological readiness level were observed using articles and the medium technological readiness level were mapped using patents. Most of the patents are generally originated from China and US, but whilst Chinese patents are appropriate only in their country, most of the technologies developed in the US are being patented for exportation. Bioremediation patents are focused mainly on water and soil bioremediation, and only a small fraction on sludge bioremediation. The most used agents are bacteria, fungi and enzymes, followed by treatments using algae, plants, and protozoa. Mutation and genetic engineering processes are still a recent trend in bioremediation.

One of the weaknesses of bioremediation can be the barriers to import or export microorganisms-based technologies.

A recent trend in oil bioremediation of soils is the combination of bioremediation agents (fungi and bacteria) in the same process, thus making the process more robust to environment changes, being a potential research opportunity.

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Table 3
SWOT matrix showing strengths, weakness, opportunities, and treats of bioremediation technologies.

Strengths ● Technologies may be used in diverse bioremediation matrices and for a wide range of contaminants, allowing the exploration of several markets; ● Environmental sustainability in line with environmental laws to preserve the environment; ● Biodegradable co-products and by-products; ● High degradation power of pathogenic compounds; more quickly and efficiently compared to other existing processes; ● Acts aerobically or anaerobically; ● Resistant to high temperatures and high salinity; ● Potential for association with other bioremediation agents to improove results; ● Use of by-products from other industries as initial food for microorganisms.	Weakness ● Technologies know-how oriented; ● Customer-made technologies that may not work properly in other environments; ● Maintain microorganism conditions during transportation.
Opportunities ● Worldwide awareness of more environmentally friendly technologies; ● Recover productive land capacity to plant crops; ● New unexplored technologies opening new market opportunities; ● Several technologies in the public domain in various countries that are already available for use due to filings only in the priority country; ● Genetic engineering still not widely explored; ● Environmental policies and norms, increasing worldwide, focused on environmentally friendly technologies; ● Transnational companies interested in bioremediation technologies; ● Worldwide awareness of environmental sustainability.	Treats ● International laws framework and barriers to import or export; ● Microorganism technologies are still in their early years and may have future import/export barriers; ● Low supply of raw material for inputs in the market and consequent increase in the purchase price; ● Non-internationalization of assignees, filing only in the priority country may lead to technologies developed in one country being used without royalties in another country; ● Still poorly exploited market; ● Yearly increase in the number of bioremediation patent filings may point to future fierce market competition.

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Appendix A. Supplementary data

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