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Development of Biochemically Enhanced Oil Recovery Technology for Oil Fields – A Review

Przegląd kierunków rozwoju biochemicznych metod wspomagania wydobycia ropy naftowej ze złóż ropnych

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ABSTRACT: Crude oil, a major source of energy, is being exploited as a driver of the economy throughout the world. Being a limited resource, the price of crude oil increases constantly and the exploitation of mature reservoirs becomes essential in order to meet the ever-increasing energy demands. As conventional recovery methods are not sufficient to fulfil the growing needs, there is an incessant demand for developing new technologies which can help in efficient tertiary recovery in old reservoirs. Petroleum biotechnology has been emerging as a branch that can provide solutions to major problems in the oil industry, including increasing oil production from marginal oil wells. The enhanced oil recovery (EOR) method comprises four methods – chemical, thermal, miscible, and immiscible gas flooding – as well as microbial interference to increase recovery of the remaining hydrocarbons trapped in reservoir rocks. Biochemically enhanced oil recovery comprises an array of blooming technologies for tertiary oil recovery methods which is eco-friendly, cost-effective, and efficient in extracting the residual oil trapped in reservoir rocks. Biochemical enhanced oil recovery (BcEOR) is based on the principle of using biochemical by-products produced by microbial species to enhance oil recovery, etc. All these technologies work on the principles of reducing viscosity, increasing permeability, modifying solid surfaces, emulsifying through adherence to hydrocarbons, and lowering interfacial tension. BcEOR technologies either employ the beneficial microorganism itself or the biochemical by-products produced by the microbial species to enhance tertiary oil recovery. This review paper discusses the chronological development of biologically enhanced oil recovery and its various mechanisms.

Key words: enhanced oil recovery, polymer flooding, biopolymers, bioplugging, oil fields, BcEOR.

STRESZCZENIE: Ropa naftowa jest wykorzystywana na całym świecie jako główne źródło energii. Ze względu na ograniczone zasoby ropy naftowej jej cena stale rośnie, a eksploatacja ze złóż dojrzałych staje się niezbędna do zaspokojenia ciągle zwiększającego się zapotrzebowania na energię. Ponieważ konwencjonalne metody wydobycia nie wystarczają do zaspokojenia coraz większych potrzeb, istnieje nieustanne zapotrzebowanie na rozwój nowych technologii, które mogą pomóc w efektywnym wspomaganiu wydobycia ze starych złóż metodami trzecimi. Ostatnio biotechnologia naftowa staje się gałęzią, która dostarcza rozwiązań dotyczących głównych problemów przemysłu naftowego, w tym zwiększenia wydobycia ropy z brzeżnych odwiertów ropnych. Wspomaganie wydobycia ropy naftowej (EOR) obejmuje cztery rodzaje metod: chemiczne, termiczne, tzw. mieszające i niemieszające wypieranie ropy gazem, a także oddziaływanie mikrobiologiczne w celu zwiększenia wydobycia węglowodorów uwięzionych w skałach złożowych. Biochemiczne metody wspomagania wydobycia ropy naftowej to szereg rozwijających się technologii dla trzecich metod wspomagania wydobycia, które są przyjazne dla środowiska, racjonalne pod względem kosztów i efektywne, jeżeli chodzi o wydobycie ropy rezydualnej uwięzionej w skałach złożowych. Biochemiczne wspomaganie wydobycia ropy naftowej (BcEOR) oparte jest na zasadzie, zgodnie z którą biochemiczne produkty uboczne wytwarzane przez gatunki drobnoustrojów są wykorzystywane do wspomagania wydobycia ropy naftowej itp. Wszystkie te technologie działają na takich zasadach jak: zmniejszenie lepkości, zwiększenie przepuszczalności, modyfikacja powierzchni ciał stałych, emulgowanie poprzez adhezję do węglowodorów, obniżenie napięcia międzyfazowego. Technologie BcEOR albo wykorzystują pożyteczny mikroorganizm jako taki, albo wykorzystują biochemiczne produkty uboczne wytwarzane przez gatunki drobnoustrojów w trzecich metodach wspomagania wydobycia ropy naftowej. W niniejszym artykule przeglądowym omówiono chronologiczny rozwój biologicznych metod wspomagania wydobycia ropy naftowej i ich różne mechanizmy.

Słowa kluczowe: wspomagane wydobycie ropy naftowej, nawadnianie z zastosowaniem polimeru, biopolimery, blokowanie z użyciem mikroorganizmów, złoża ropne, biochemiczne wspomaganie wydobycia ropy naftowej (BcEOR).

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Introduction

The major source of global energy supply continues to be fossil fuels, which contribute approximately 85% of our total energy needs (34% oil, 24% natural gas, and 27% coal) (Statistical Review of World Energy, 2019). As oil is the key player in meeting world energy demands with fossil fuels, enhanced recovery of oil is becoming a major challenge for the oil industry around the globe. Therefore, it is necessary to make oil production more efficient, sustainable, and green. Recently, petroleum biotechnology has emerged as a branch that can provide solutions to major problems in the oil industry, including increasing oil production from marginal oil wells (non-producing wells) (Montiel et al., 2009).

In general during the first stage of oil production, the differential pressure between a reservoir and a wellbore is responsible for driving oil out of the production well. This process recovers only about 10% of the original oil in place (OOIP) and is referred to as primary production. Later, with a decline in reservoir pressure, oil recovery also decreases, leading to the need for secondary recovery. Secondary recovery involves the injection of an external fluid (such as water and gas) through the injection wells to maintain reservoir pressure and displace the oil towards the wellbore (Zendehboudi and Bahadori, 2017). During this process, the water physically sweeps the oil, which produces 15–60% of the OOIP. Petroleum industries are aware of the inefficient oil recovery inherent in the conventional means of primary and secondary recovery. Therefore, the oil industries further adopted the enhanced oil recovery (EOR) process to increase oil production by improving oil flow and sweep efficiency in the reservoir. Since then, several methods have been developed to improve the sweep efficiency of oil by increasing the mobility ratio, displacing the oil for enhanced recovery (Thomas, 2007). The EOR method comprises four methods – chemical, thermal, miscible, and immiscible gas flooding – as well as microbial means of increasing recovery of the remaining oil (Planckaert, 2005).

Over the past 40 years, polymer flooding has been carried out in marginal oil fields and has proved to be successful in many cases. Polymer flooding operates on the principle of chemical recovery (da Silva et al., 2007). It involves the addition of a polymer (a viscosifying agent) to the injected water, which tends to increase water viscosity, thereby increasing the mobility of the water-to-oil ratio. However, earlier laboratory and field trials revealed that salinity and temperature are the major issues that lead to polymer degradation and adsorption on the rock surface. Microbial degradation and concentration are also major issues leading to a loss of viscosity and pore throat plugging. Enhanced oil recovery using polymer flooding and microbial enhanced oil recovery can act synergistically to solve the issues that limit efficient recovery process. In order to successfully implement enhanced oil recovery, many groups of researchers have experimentally investigated the feasibility and potential of using novel biopolymers developed through synergistic chemical and microbial technology (BcEOR) (Yen, 1990; Lazar et al., 2007; Zhang and Xiang, 2010; Shibulal et al., 2014, 2018; Cui et al., 2019).

In this review, we provide an update on the chronological development of biologically enhanced oil recovery, the various mechanisms involved, and its advantages and disadvantages. This comprehensive review provides better insight to increase the efficiency of the oil recovery process in order to further improve the available processes in future.

History of biologically enhanced oil recovery

Microbial enhanced oil recovery (MEOR) is a collection of techniques that utilise microorganisms and their metabolic products to improve the recovery of crude oil from reservoir rock (Yen, 1990; Lazar et al., 2007; Zhang and Xiang, 2010; Shibulal et al., 2014, 2018; Cui et al., 2019). The recovery can either be in the form of cyclic (single-well simulation), microbial flooding, or selective plugging recovery (Lazar et al., 2007; Shibulal et al., 2014). The idea of microbial enhanced oil recovery was first proposed by Beckmann (1926), when he published results on the possibility of using microbial metabolic processes to improve the oil production rate. In the later parts of the 1940s, the experiments of Zobell (1947) further indicated the potential for microbial oil recovery from sand grains. The study highlighted the similarity between the compounds used to improve water flood efficiency in chemical and miscible EOR processes and the products of microbial fermentation of carbohydrates – despite the setback due to hydrogen sulphide production.

From the classic works of Beckmann (1926) and Zobell (1947), there was a giant leap from the 1950s through the 1980s, with other scientists reporting advances made in MEOR (Updegraff and Wren, 1954; Kuznetsov, 1961; Kuznetov et al., 1962; Senyukov et al., 1970; Lazar, 1978; Ivanov et al., 1982; Belyaev, 1983; Bubela, 1983; Grula et al., 1983; Yarbrough and Coty, 1983; Zajic et al., 1983; Donaldson and Grula, 1985). Further studies were conducted in the 1990s and 2000s with renewed significant interests (Lazar, 1991; Ivanov et al., 1993; Hitzman and Sperl, 1994; McInerney and Sublette, 1997; Bryant and Lockhart, 2002; Li et al., 2002; Maudgalya et al., 2005). A parallel development was the rise in crude oil prices due to the petroleum crisis in the 1970s that boosted development of MEOR research and validated it to scientific enhanced oil recovery method (Lazar et al., 2007).

Table 1. Some of the major field trials around the globe
Tabela 1. Niektóre z ważniejszych prób na złożach na świecie

Country	Microbial systems used	Result of oil production	References
Bulgaria	– Indigenous oil-oxidising bacteria from water injection and water formation	Positive	Groudeva et al., 1993
Canada	– Pure culture of *Leuconostoc mesenteroides*	Negative	Jack and Stehmeier, 1988
Former East Germany	– Mixed cultures of thermophilic *Bacillus* and *Clostridium*	Positive	Wagner et al. 1993
USA	– Pure or mixed cultures of *Bacillus*, *Clostridium*, and *Pseudomonas* – Mixed cultures of hydrocarbon-degrading bacteria – Slime-forming bacteria	Positive	Hitzman, 1983, Grula et al., 1983, Bryant et al., 1993, Jenneman et al., 1995, Dietrich et al., 1996
Russia	– Pure culture of *Clostridium pyrobutyricum* – Bacteria mixed cultures – Indigenous microflora of water injection and water formation	Positive	Senyukov et al., 1970, Ivanov et al., 1993, Wagner and Lungerhausen, 1995
China	– Mixed enriched bacterial cultures of *Bacillus*, *Pseudomonas*, *Bacteroides*, etc. – Slime-forming bacteria – Biopolymers and biosurfactants – Stimulation of indigenous microbes	Positive	Wang et al., 1993, Cui et al., 2019
Romania	– Adapted mixed enriched cultures; *Clostridium*, *Bacillus*, and *Pseudomonas*	Positive	Lazar and Constantinescu, 1985, Lazar, 1991, 1998
Poland	– Mixed bacteria cultures	Positive	Karaskiewicz, 1975

There are numerous examples of MEOR being applied in different oilfields around the globe. To list all these examples would be an enormous task, but some of the best known cases are presented in Table 1. Currently there are other ongoing MEOR projects in different parts of the world. In the North Sea, out of the 19 enhanced oil recovery projects underway in 2006, only one used microbial enhanced oil recovery (Awan et al., 2008); the other 18 projects have been or are gas enhanced oil recovery projects.

In general, with an average of 35–45% recovery from the best currently available technology of the OOIP in an oil field coupled with an annual production declines of between 4–15% in mature fields, many more oil companies and agencies are opening up to the possibility of using MEOR permanently. It is believed that the use of MEOR will continue to grow over time, as the basic processes involved in MEOR become better understood.

Gasses, solvents, surface active compounds, polymers, organic acids, and biomass are all regular and predictable products of microbial metabolism similar to compounds used in chemical enhanced oil recovery (Sheehy, 1991). Microbial enhanced oil recovery in general has many advantages, such as cost-effectiveness, low toxicity, biodegradability, biocompatibility, and selectivity and specificity (Desai and Banat, 1997). MEOR, therefore, offers a good alternative in improving the recovery of crude oil from reservoirs by utilising microorganisms and their metabolic products.

The continual search for a cheaper and more effective EOR method was a major driving force behind the development of the microbial technique. The advances that were made in the 1950s through the 2000s came in large part from a great deal of work studying how microorganisms can benefit the recovery of oil from petroleum reservoirs. Many of the results from laboratory studies were promising. The laboratory study of a specific microorganism was done either for the surface production of various compounds or for the injection of cells into a reservoir for *in situ* production of metabolic compounds. These laboratory studies on MEOR normally used core samples and columns containing the desired substrates. These substrates were employed to demonstrate the usefulness of biosurfactants in oil recovery from sandstone and carbonate. Similarly, core samples were used as a model for the movement of microorganisms and nutrients through substrates in order to ascertain their usefulness after injection into oil reservoirs (Banat, 1995).

However, the results from field applications were mixed because the biological, chemical, and physical processes that occur in petroleum reservoirs where *in situ* metabolism occurs were not fully understood (Donaldson, 1991). As observed by Hitzman (1991), several reasons can be considered for the reported differences between laboratory results and field observations in MEOR studies. One of the important factors is the dynamic environment normally encountered in a reservoir, which is difficult to duplicate or simulate in a laboratory with small cores and reactors. Physical and chemical changes also occur within the reservoir as a result of interactions between the multiplying microorganisms and the reservoir matrix that cannot be duplicated in the laboratory. Another major reason identified for the failure of field trials is insufficient consideration of the conditions which characterise petroleum reservoirs (Sheehy, 1991). Sheehy observed that the activity of bacteria in reservoirs depends on the physical and chemical conditions they encounter. These include pH, temperature, salinity, pressure, ionic strength, source of energy, and nutrients. Moreover, the lack of adequate knowledge about the growth of microorganisms in oil under anaerobic conditions during the early days of MEOR was a major factor. It was not until recently that bacteria have been shown conclusively to metabolise hydrocarbons in oil in an anaerobic environment (Kropp et al., 2000). However, it was suggested that some of the perceived technical problems associated with MEOR applications can be overcome with careful planning (Moses, 1991; Maudgalya et al., 2007).

In spite of some of the earlier setbacks, MEOR has developed rapidly over the past two or three decades around the world. From the USA to Russia, Europe to China, and Canada to Australia, several studies have been carried out in different applications of MEOR: for example, Senyukov et al., 1970; Dienes and Jaranyi, 1973; Karaskiewicz, 1975; Lazar, 1978; Yarbrough and Coty, 1983; Hitzman, 1988; Sheehy, 1991; Wagner, 1991; Ivanov et al., 1993; Wang et al., 1993; He et al., 2000; Bryant and Lockhart, 2002; Li et al., 2002). The first field trial was carried out in 1954, in the Lisbon field of Union County, AR (Yarbrough and Coty, 1983). The field tests from many of these studies specified the injection of mixed anaerobic or facultative anaerobic bacteria, typically consisting of *Clostridium*, *Bacillus*, *Pseudomonas*, *Micrococcus*, *Mycobacterium*, *Arthrobacterium*, *Peptococcus*, etc., along with nutrients. One example of such a nutrient is molasses, a by-product of sugar, which is relatively inexpensive in that part of the world. The selection of these microorganisms is based on their ability to generate high quantities of gasses (e.g. CH_4, H_2, CO_2, and N_2), organic acids (e.g. butyric and acetic acids), solvents (e.g. acetone, butanol, and ethanol), polymers (e.g. polysaccharides), biosurfactants, and cell biomass. Each mechanism or combination of these mechanisms could lead to increased oil recovery (McInerney et al., 2002).

Research carried out from 1970 to 2000, as illustrated in the studies by Lazar et al. (2007) and Brown (2010), has established the basic nature and existence of indigenous microbiota in oil reservoirs, as well as reservoir characteristics being essential to a successful MEOR application. At the moment, research into MEOR is still continuing, which can be said to be the fourth generation of studies. This is buoyed by the combined effects of increasing mature oilfields and rising oil prices, as well as the need to increase our understanding of MEOR processes – as many of the earlier studies identified the need to improve critical information on the mechanisms, metabolic rates, and required concentrations of microbial products. Some of the most recent works include those of Brown et al. (2002), Bryant and Lockhart (2002), Maudgalya et al. (2005), Kowalewski et al. (2006), Kaster et al. (2009), Jimoh et al. (2011), Rudyk and Sřgaard (2011), and several other studies. All these works were attempts to bridge the gap in laboratory success and the field applications of MEOR.

The research showed that there has been improvement in the availability of methods and analytical equipment, among other things. Also, new strains of bacteria have been identified and isolated from deep-seated reservoirs that have the ability to grow in extreme salinity and temperatures. Examples of such newly identified strains of bacteria include thermoanerobic bacteria, such as *Thermoanerobacter brockii* subsp. *lactiethylicus* strain 9801T – which was isolated from a deep subsurface French oil well at a depth of 2,100 m, where the temperature was 92°C and optimum growth at temperatures between 55 and 60°C (Cayol et al., 1995) – and *Thermoanaerobacter tengcongensis* strain MB4T, isolated from a Chinese hot spring capable of growing at temperatures between 50 and 80°C (Xue et al., 2001).

Moreover, the area of MEOR modelling is also improving. It was recognised that a mathematical model could be used to recognise the most important parameters and their practical relationships for the application of MEOR (Marshall, 2008). However, developing detailed mathematical models for MEOR is an extremely challenging task, not only as a consequence of the natural difficulty of working with the microbes, but also because of the diversity of physical and chemical variables that control bacterial activity in subsurface porous media. Microbial modelling developed from the earlier work of Monod (1949), which modelled the bacteria growth in several mathematical models that simulated MEOR processes. Examples include models for the multidimensional flow of a multiphase fluid consisting of water and oil in a porous medium, along with specific equations for absorption, adsorption, and diffusion of metabolites, microorganisms, and nutrients (Chang et al., 1991; Islam, 1990), models for relative permeability changes (Al-Wahaibi et al., 2006; Nielson et al., 2010), and models that incorporate salinity, the effects of adsorption of microorganisms, reduction of interfacial tension, and wettability changes (Behesht et al., 2008).

In conclusion, microorganisms have the ability to enhance oil recovery by virtue of some of the products they can produce (Brown, 2010), or specifically employing this ability in an economical, practical, and scientifically valid manner, transferring it from a laboratory scale to large-scale field applications. More research is required in this area and it is believed that by doing so, MEOR – as part of tertiary enhanced oil recovery methods – could substantially increase the world's supply of oil.

The mechanisms involved

Over the years attempts have been made to classify the main mechanisms involve in the MEOR process. These processes are identified based on the end products generated from bacterial metabolism. According to Janshekar (1985), the main mechanisms of MEOR include viscosity reduction, rock dissolution, permeability reduction, etc. (Fig. 1). All these mechanisms are similar to those being practiced in chemical EOR. The main difference is that the required products come from bacterial metabolism. It is therefore expected that the MEOR mechanisms fulfil the basic law of thermodynamics. The MEOR mechanisms, however, can be different from bacterium to bacterium, and are normally selected based on the requirements of the wells or reservoir.

It has been shown that MEOR techniques are generally applied to reservoirs where production rates have declined over time. The reasons behind the consideration of MEOR technologies (Hitzman, 1991) when evaluating reservoirs for residual oil recovery usually include multiple application possibilities, multiple oil recovery mechanisms, increased treatment effectiveness with penetration and duplication, and low start-up costs along with low operating costs.

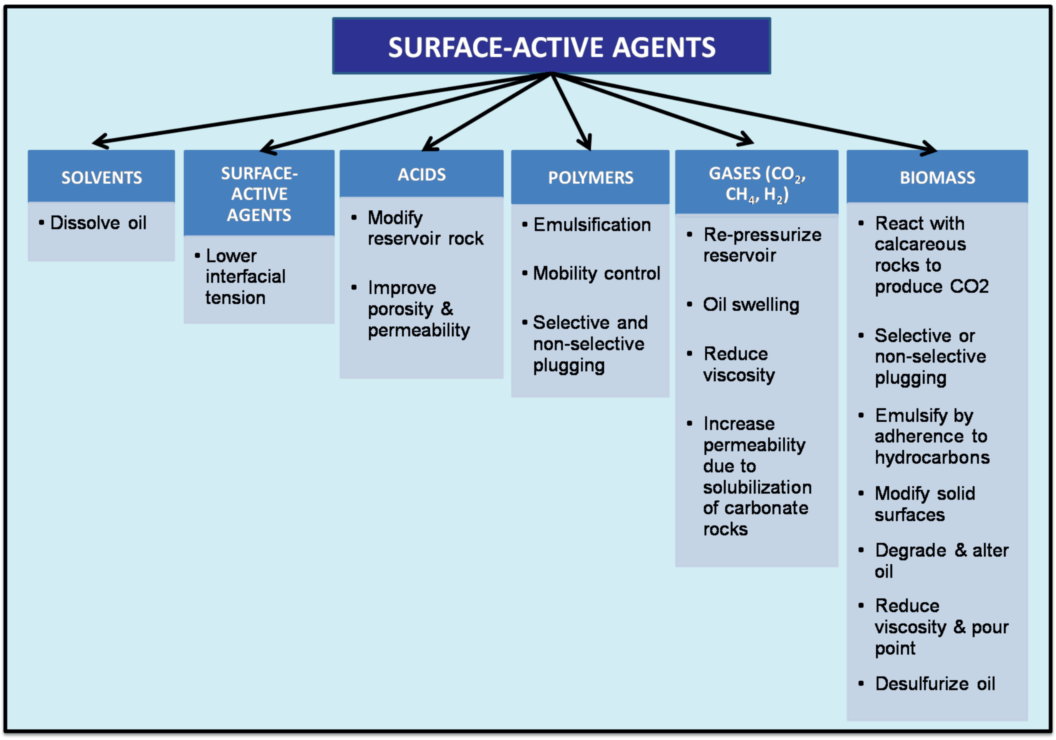

Fig. 1. Microbial products and their contribution to enhanced oil recovery

Rys. 1. Produkty mikrobiologiczne i ich udział we wspomaganiu wydobycia ropy naftowej

The MEOR methods are believed to be more constructive than other EOR methods based on the perceived advantages described above; moreover, the microbes produce the necessary metabolites *in situ*, the method is considered to be environmentally friendly, and it does not require large amounts of energy.

The various mechanisms mentioned in Figure 1 work synergistically to change the reservoir chemistry at the micro-environmental level, which in return enhances the free flow of entrapped oil and finally increases the recovery of hydrocarbons from depleting wells. The different mechanisms have different impacts on reservoir chemistry *viz.* the biodegradation of large molecules reduces the viscosity of hydrocarbons and the production of bio-surfactants reduces interfacial tension. Similarly, the production of various gasses builds additional pressure inside the well, which acts as a driving force to drift the oil towards the surface. Some of the microbial metabolites may reduce permeability by activating secondary flow paths. The enhanced growth of nitrate-reducing bacteria competes for food with the sulphate-reducing bacteria, causing a reduction in H_2S concentration which in turn mitigates downhole corrosion caused by sulphate-reducing bacteria, acid-producing bacteria, etc. The attachment of bacteria and the development of slime, i.e. extracellular polymeric substances, favour the plugging of highly permeable zones (thieves zones), leading to increased sweep efficiency of otherwise unswept oil.

Classification of BcEOR

The objective of most MEOR is to reduce the remaining oil in the reservoir; however, the implementation of a BcEOR strategy may be different. Nevertheless, two major strategies are normally employed in BcEOR. The first one is the injection of bacteria and nutrients, normally referred to as the 'traditional' MEOR method, whilst the second method involves the stimulation of indigenous bacteria through the injection of nutrients. The application of MEOR technology can either be in the form of a cyclic (single-well simulation), microbial flooding, or selective plugging recovery (Lazar et al, 2007).

In cyclic microbial recovery, microorganisms and nutrients are injected into production wells. The wells are shut in for a long enough period to allow microbial growth and metabolite formation. This can take a number of days or weeks. Finally, the oil production phase begins and extends over a period of weeks or months. In cyclic microbial recovery, when production declines, another phase of injection is normally started. In this case, the depth of the area covered by bacteria would be limited by the injection rate and the kinetics of the microbial process (Bryant and Lockhart, 2002).

The second type of application is microbial flooding, in which the microbial growth is usually stimulated by adding nutrients to the injection water to encourage the proliferation of microorganisms which are indigenous to the formation. If the requisite microbial activity is not present, then microorganisms can be injected into the formation along with the nutrients. In some approaches, injection into the formation is stopped to allow time for the *in situ* growth and metabolism to occur (Youssef et al., 2009). In other approaches, brine injection continues after nutrient and/or cell injection. This option would most likely be less expensive, as the growth would be stimulated in larger parts of the reservoir, particularly where the carbon source (residual oil) is located, which is usually the target of the enhanced oil recovery treatment (Kaster et al., 2012).

Microbial selective plugging encompasses a microbial process to divert water into low-permeability regions to block water channels deep in the reservoirs. With this type of treatment, nutrient preferentially flows into the high-permeability regions, which then stimulates biomass and polymer production in these regions, both of which reduce the permeability of the rock (Raiders et al., 1985). In contrast, heavy oil modification is usually accomplished by microbial decomposition of long chain compounds within the formation.

Microbe selection for BcEOR

Many microbes have the ability to produce secondary metabolites which can enhance oil recovery, such as acids (*Clostridium* sp. or *Enterobacter*), gasses (*Enterobacter* or *Clostridium* sp.), solvents (*Clostridium acetobutylicum* or *Zymomomas mobile*), biomass (*Bacillus lichenifonnis* or *Xanthomonas campestris*), biosurfactants (*Acinetobacter calcoacticus, Arthrobacter paraffineus* or *Pseudomonas* sp.), and biopolymers (*Bacillus polymyxa* or *Brevibacterium viscogenes*). The microbial bioproducts will determine the choice of bacteria to increase oil recovery and withstand the extreme reservoir conditions. In the case of MEOR, the successful field experiments have mostly used anaerobic bacteria (Maudgalya et al., 2007) and four main sources from which bacterial species that are potential candidates for MEOR can be isolated have been suggested: formation waters, sediment from formation water purification plants, sludge from biogas operations, and effluents from sugars (Lazar, 1991). A generalised workflow for the selection and isolation of BcEOR microbial strains is depicted blow (Fig. 2).

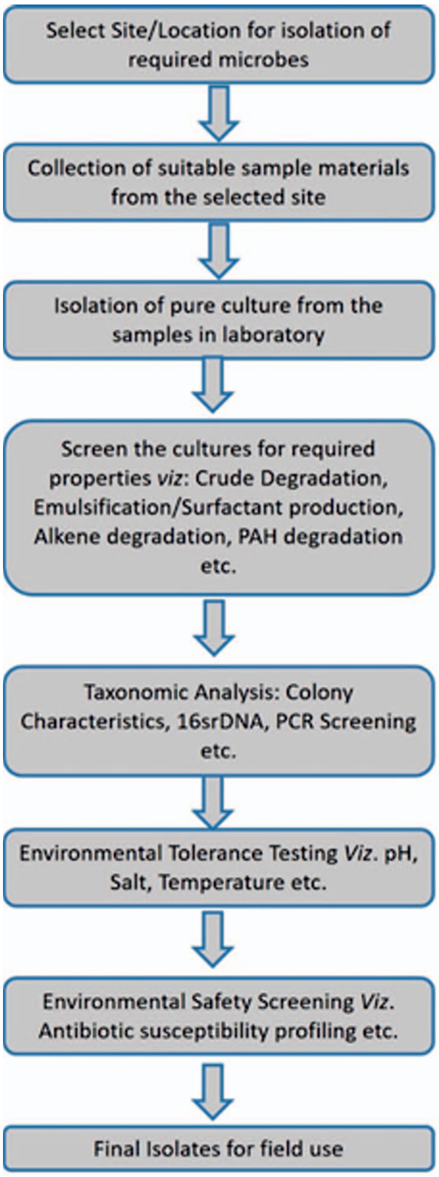

Fig. 2. Generalised workflow for the selection and isolation of BcEOR microbial strains

Rys. 2. Zgeneralizowany schemat postępowania w przypadku selekcji i izolacji szczepów mikroorganizmów stosowanych w metodzie biochemicznego zwiększenia wydobycia ropy naftowej (BcMEOR)

Earlier studies on MEOR showed that both mixed cultures and pure strains of bacteria have been used. For example, Hitzman (1983) used pure and mixed cultures of *Bacillus*, *Clostridium*, and *Pseudomonas* in 2–4% molasses in the USA. Wang et al. (1993, 1995) used mixed enriched bacterial cultures of *Bacillus*, *Pseudomonas*, *Eurobacterium*, *Fusobacterium*, and *Bacteriodes* in a 4% residue sugar. Dostalek et al. (1957) and Dostalek and Spurny (1958) injected sulphate-reducing *Desulfovibrio* and hydrocarbon-utilising *Pseudomonas* bacteria with nutrients (generally molasses). Yaranyi (1968) documented the use of mixed sewage sludge bacterial cultures, predominantly *Clostridium*, *Pseudomonas*, and *Desulfovibro*. Karaskiewicz (1975), in 18 field trials in Poland between 1960 and 1961, also documented the use of mixed microbial cultures containing *Pseudomonas*, *Escherichia*, *Arthrobacter*, *Mycobacterium*, *Micrococcus*, *Peptococcus*, *Bacillus*, and *Clostridium* which were grown in formation water and were injected along with 4% molasses. Further studies that used mixed cultures of bacteria include those of Wagner et al. (1993) that employed mixed cultures of thermophilic *Bacillus* and *Clostridium*, mixed cultures of hydrocarbon-degrading bacteria in free corn syrup and mineral salts were used by Coates et al. (1993), Nelson and Schneider (1993), Jenneman et al. (1995), and others.

Some studies employed pure strains of bacteria, such as the one by Wagner and Lungerhausen (1995), who used salt-tolerant *Clostridium* to increase oil production in a carbonate reservoir by *in situ* gas and solvent production. Also, Grula et al. (1983) used isolated Clostridia species that were able to produce solvents and gasses. Furthermore, Ivanov et al. (1993), Nazina et al. (1994), Belyaev et al. (2004), and Jimoh et al. (2011), all used pure cultures of *Clostridium tyrobutyricum* in 2–6% molasses for different evaluations of MEOR processes.

BcEOR constraints

A major reason for the failure of BcEOR technology is insufficient consideration of the conditions which characterise petroleum reservoirs and the physiology of the microorganisms which thrive in these conditions (Sheehy, 1991). The activities of microbes employed in the BcEOR process depends on the physical and chemical conditions they encounter in the reservoirs – temperature, pressure, pH, salinity, redox potential, etc. – although these reservoir conditions vary a great deal from one reservoir to another. All these factors, which are mostly physical and environmental, can affect the growth, proliferation, metabolism, and survival of bacteria and can limit their ability to produce the quantities of metabolites necessary for EOR. However, the general opinion is that with proper planning most of these factors can be overcome. Some of the factors which are considered limiting factors for successful application of BcEOR are enumerated below.

Temperature

Temperature plays a significant role in bacterial metabolism. Temperatures rise with increasing depth. Therefore, it is certain that bacterial growth and metabolism will be affected, as higher temperatures can exert negative effects on enzyme function by disrupting important cell activities. This molecular picture of the effects of temperature on enzyme function is generally accepted, but it has also been observed that the temperatures at which these phenomena occur vary widely between organisms (Marshall, 2008). Microbes can be categorised, according to the optimal temperature ranges for their survival, into psychrophiles (<25°C), mesophiles (25–45°C), and thermopiles (45–60°C).

The depths at which most oil reservoirs are situated have temperatures higher than 37°C, which is considered the optimum temperature for most bacteria. For example, in the North Sea the temperature gradient is about 2.5°C/100 m (Vermooten et al., 2004); therefore, at a depth of 3000 m, the temperature can reach as high as 90°C.

Pressure

Pressure affects biological processes in relation to the accompanying volume changes; however, in many regions of the earth, the limiting boundary is probably set more by high temperatures than by high pressures (Marquis, 1976, 1983). The maximum depth for life in the deep earth has not been determined, but for maximum recovery of oil – in the range of 2000 to 3000 meters – the most applicable pressures for EOR in productive wells are 20 to 30 MPa. High hydrostatic pressures of dozens of MPa are generally assumed to be non-lethal, but can exert adverse effects on the growth of organisms that are adapted to atmospheric pressure (Abe et al., 1999; Bartlett, 2002).

The effect of pressure on microorganisms depends not only on the magnitude of pressure, but also on the duration for which it is applied in combination with the temperature, pH, oxygen supply, and composition of the culture media (Abe, 2007). The effects of pressure can be very complex and often difficult to interpret. For example, recent results indicated that lactic acid bacteria (*Lactobacillus sanfranciscensis*) growth at 50 MPa was 30% lower than at atmospheric pressure and that an increase in temperature did not improve its piezotolerance (Molina-Höppner et al., 2003). In another study, it was shown that treatment of *E. coli* cells at a higher pressure (75 MPa) for 30 min did not readily cause any morphological changes (Kawarai et al., 2004). The challenges are therefore to establish whether the physiological responses of bacterial cells to high pressure are relevant to their growth and to identify the critical factors in cell viability and lethality under high pressure during MEOR.

pH

pH is one of the major environmental factors that affect microbial growth and is one of the most studied because of its importance in fundamental research. In general, the optimal pH for microorganism growth is between 4.0 and 9.0, but at very low pH the metabolic activities of microorganisms can be affected. The detrimental effect of low pH on microbial growth is well-documented (Brock, 1969) although the mechanisms involved are not well understood. Generally, a near-neutral intracellular pH is maintained in bacteria (Riebeling et al., 1975), but the intracellular pH can decrease considerably if the cell is subjected to an acidic environment.

As many enzymes are sensitive to pH, the growth inhibitions seen at low pH could be caused by a direct effect of the H ion on cellular components, even though such direct effects would not necessarily cause a decrease in the efficiency of growth (Russell and Dombrowski, 1980). pH values normally encountered in oil reservoirs may not pose a problem for the growth of organisms, but pH gradients can affect the control of specific metabolic processes required for some MEOR processes (Jenneman and Clark, 1992).

Salinity

Sodium chloride makes up about 90% or more of the total dissolved solids found in reservoir brines; therefore, microorganisms' tolerance to salt concentration is one of the most important characteristics for microorganisms used in MEOR. The extent to which salinity causes changes in bacterial growth and metabolism depends on the osmotic balance required for such growth, since the solute concentration of the surrounding environment can affect cell growth. Grula et al. (1983) isolated Clostridia species capable of growing at 45°C, but found that their ability to produce solvents and gasses was reduced significantly at high sodium chloride concentrations (5% w/v).

General concentrations of oilfield brines can vary from 100 mg/l to over 300 g/l (Gran et al., 1992) and the salinity gradient can be different in the range of the same formation . Most bacteria overcome osmotic stress by accumulating organic compatible solutes within the cytoplasm without the need for changing intracellular proteins. This method is called the 'organic osmolyte strategy' (Roberts, 2005). The second adaptation strategy is intracellular accumulation of high concentrations of K+ (Oren, 2001).

Pore size

Even though the pores in rock can be connected in different ways, pore spaces less than 0.5 nm can place severe restrictions on the ability of most bacteria (most bacteria have lengths of approximately 0.5–10.0 um and widths of 0.5–2.0 um) to be transported through the rock matrix, especially for bacteria whose sizes are comparable to those of the rock pores (Jenneman and Clark, 1992). Updegraff (1983) stated that pores must be at least twice the diameter of cocci or short bacilli for effective transport to occur. Fredrickson et al. (1997) also showed that the sizes of pores within the rock, or the pore throat diameter, may be an important factor in regulating the observed microbial activity.

Pore throat diameters of shale are on average much smaller than those of sandstone (~0.2 mm for shale and up to 13 mm for sandstone) (Krumholz, 2000), and the results of the study suggest that the growth and metabolism of shale-bound organisms may be limited by the slow diffusion of nutrients and/or the inability of microbes to migrate easily through the narrow pores. Also, Zvyagintsev (1970) in an experiment with microbes, stated that placing microbes in large capillaries (400 × 150 nm) increased the number of cells 7–10 times, but in small capillaries not only was an increase of cells observed, but the size of the cells was reduced. In general, a permeability of 75–100 mD is thought to be the lower limit for effective microbial transport (Jenneman and Clark, 1992), but reports have indicated transportation of bacterial cells through cores of less than 75 mD (Hart et al., 1960; Kalish et al., 1964).

Nutrients

A successful MEOR process will require the availability of essential nutrients in order for growth and metabolism to take place, as it was recognised that there is a smooth relationship between growth rate and nutrient concentration (Monod, 1949). Bacterial requirements for growth include sources of energy, mostly organic carbon (i.e. sugars and fatty acids) and mineral ions (e.g. iron and phosphorus). These nutrients are mostly transported in the aqueous phase. Fermentative bacteria use nutrients containing glucose, sucrose, or lactose.

The choice of nutrients is very important since the types of bioproducts that are also produced by different types of bacteria are dependent on the types, concentrations, and components of the nutrients provided. Molasses in general has been employed as the carbon source in many of the field applications because of its price and essential mineral and vitamin content. The use of molasses as a substrate was first proposed by Updegraff and Wren (1954). In addition, some microbes utilise oil as a carbon source, which is excellent for heavy oil production because it can reduce the carbon chain of heavy oil and increase the quality (Cooper et al., 1980; Moses, 1991). Under anaerobic conditions, however, the use of petroleum components as food is thought to be ineffective, at least within the timeframe required for economic recovery. Even though growth can occur, the growth can be very slow and hardly detectable for several months (Moses et al., 1993).

Advantages of BcEOR

In many ways BcEOR is a more advantageous technology than the other prevailing technologies. The first and foremost advantage of BcEOR technology is its environmentally friendly and sustainable nature. The bacterial strains used for an installation are native to it, so the threat of ecological imbalance is nullified. Moreover, the nutrients used are inexpensive and easy to obtain. This technology is economically attractive for marginally producing oil fields and a suitable alternative to abandoning marginal wells. According to a statistical evaluation (1995 in the USA), 81% of all BcEOR/MEOR projects demonstrated a positive incremental increase in oil production and no decrease in oil production (Lazar et al., 2007). Only minor modifications to the existing field facilities are necessary to implement the BcEOR process. The effects of bacterial activity within the reservoir are magnified by their growth, whilst other EOR technologies have additive effects that decrease over time and distance. Moreover, BcEOR processes are particularly suited for carbonate oil reservoirs where some EOR technologies cannot be applied efficiently. Most importantly, BcEOR products are all biodegradable and will not accumulate in the environment, so this technology is highly sustainable and environmentally friendly.

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