TOXICITY STUDY OF ISO-ELECTRIC FOCUSING IN LABORATORY TO PHARMACY VALUATION

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Abstract

Nucleic acid molecules are separated by applying an electric field to move the negatively charged molecules through anagarose matrix. Shorter molecules move faster and migrate farther than longer ones because shorter molecules migrate more easily through the pores of the gel. This phenomenon is called sieving. Proteins are separated by charge in agarose because the pores of the gel are too large to sieve proteins. Gel electrophoresis can also be used for separation of nanoparticles.

Keywords: Pharmacy, Toxicity

Mapping environmental hazards

There are many environmental health mapping tools. TOXMAP is a Geographic Information System (GIS) from the Division of Specialized Information Services of the United States National Library of Medicine (NLM) that uses maps of the United States to help users visually explore data from the United States Environmental Protection Agency's (EPA) Toxics Release Inventory and Superfund programs. TOXMAP is a resource funded by the US Federal Government. TOXMAP's chemical and environmental health information is taken from NLM's Toxicology Data Network (TOXNET) and PubMed, and from other authoritative sources.

Aquatic toxicity

Aquatic toxicity testing submerges key indicator species of fish or crustacea to certain concentrations of a substance in their environment to determine the lethality level. Fish are exposed for 96 hours while crustacea are exposed for 48

Literature Review

The present paper is organized as 3 submitted articles and 5 published articles in different journals together with a review of microbial enhanced oil recovery and extended summary and future perspectives on the research conducted in this Ph.D. study. The introduction part (Chapter 1) provides brief information on the need for enhanced oil recovery including the objectives and scope of the current thesis. Chapter 2 covers the review on microbial enhanced oil recovery that gave an overview of microbial enhanced oil recovery development from early stages, the mechanism and examples of applications over the past 40 years. Chapter 3 covers the published and submitted articles that cover different aspect of microbial enhanced oil recovery such as adaptation, MEOR mechanisms, metabolites production, microbial modification of rock, gas production and evaluation, oil recovery from packed columns among others. Chapter is the conclusion and highlights the most significant results achieved and relates the findings to the current state of the art. It is mainly based on the findings and results from the articles listed in chapter but presented with an extensive knowledge of the microbial enhanced oil recovery and it also incorporates additional 5 unpublished data from laboratory studies performed as part of this thesis. The last chapter

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(Chapter 5) provides the future perspectives on microbial enhanced oil recovery based on the outcome of this study.

Microbial enhanced oil recovery is a collection of techniques that utilizes microorganisms and their metabolic products to improve the recovery of crude oil from reservoir rock (Yen, 1990, Zhang and Xiang, 2010, Lazar, 2007). The application can be either in a form of cyclic (single well simulation), microbial flooding recovery or selective plugging recovery (Lazar, 2007). The idea of microbial enhanced oil recovery was first proposed by Beckmann (1926) when he published results on the possibility to use microbial metabolic processes to improve the oil production rate. In the later parts of the 1940's experiments from 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 even though there was a setback due to hydrogen sulphide production. Gases, 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 economical, low toxicity, biodegradability and biocompatibility, selectivity and specificity (Desai and Banat, 1997). MEOR therefore, offers good alternative in improving the recovery of crude oil from reservoir utilizing microorganisms and their metabolic products. From the classical works of Beckmann (1926) and ZoeBell (1947), it was a giant leap to the 1950s through 1980s with other scientists reporting advances made in MEOR (Updegraff and Wren, 1954; Davis and Updegraff, 1954; Kuznetsov, 1961; Kuznetov et al. 1962; Senyukov et al. 1970; Lazar, 1978, Ivanov et al. 1982, Zajic et al. 1983; Belyaev 1983; Bubela, 1983, Yarbrough and Coty, 1983, Grula et al. 1983; Donaldson and Grula, 1985).

Further researches were carried out in the 1990s through 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). Hitzman (1988) published a review on MEOR field testing and also a review of many field applications of MEOR was presented by Bryant et al. (1989). Extensive body of scientific and technical understanding of the microbial enhanced oil recovery was presented by Donaldson et al. (1989). Additionally, significant 8 contributions to MEOR area were published in the proceedings of the international conference on MEOR, Donaldson (1991) and Lazar review papers (Lazar 1991; Lazar, 1998). The continuum search for a cheaper and effective enhanced oil recovery method was a major driving force behind the development of microbial technique of enhanced oil recovery. The advances that were made in the 1950s through 2000s came in a large part, from a great deal of work looking at how microorganisms can benefit the recovery of oil from petroleum reservoirs. Many of the results from the laboratory studies were promising. The laboratory study of specific microorganism is 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 have normally utilized core samples and columns containing the desired substrates.

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Materials and methods

Microorganisms

The microorganism, Thermoanaerobacter brockii subsp. lactiethylicus strain 9801T was purchased from the German Culture Collection (DSM) and it was the same strain that was originally isolated from a deep subsurface French oil well at a depth of 2100 m where the temperature was 92 oC and now deposited at DSM. The details of the oil strata and reservoirconditions have been described [7]. The cells are gram-positive, straight motile rods (0.5 by 2 to 3 microns) with flagella uniformly distributed over the entire surface of the cell. During cultivation, optimum growth occurred between 55 and 60 oC, the fermentable substrates include glucose, fructose, galactose, mannose, cellobiose, maltose sucrose etc. The products of fermentation of glucose were lactate, acetate, ethanol, hydrogen and carbon dioxide and hydrogen while the DNA base composition is 35 mol % G + C .

Determination of most suitable nutrient broth for growth of Thermoanaerobacter brockii 9801-T ll nutrients broths were prepared by weighing the accurate amount specified on the products bottles and mixed in a volumetric flask to 1000 ml. After all salts were completely dissolved they were divided into 100 ml portions in serum bottles flushed with nitrogen and autoclaved at 121oC for 20 min. The nutrient broths were later inoculated with 0.5 ml and incubated for 42 h at 50^{o}C.

Optimization of pH in the substrate

Clostridia nutrient medium and thioglycolate broth with resazurine was prepared according to the given instructions on the bottles and divided into portion where the pH was adjusted with the help of a pH Meter and a calibrated pH electrode and NaOH and HCl. For clostridium medium, the pH was adjusted to 5.00, 6.00, 6.60 (which is the original pH of the base) 7.00, 8.00, 9.00, 10.00, 11.00 and12.00 while for thioglycolate broth, the pH was adjusted to 5.00, 6.00, 7.00(which is the original pH of the base), 8.00, 9.00, 10.00, and 11.00. 100 ml of each nutrient broth was dispensed into serum bottles, purged with nitrogen for 2 min and autoclaved at 121oC. All the serums bottles were inoculated with 0.5 ml culture with a sterile needle and syringe and incubated in an anaerobic jar at 50 oC for approximately 42 h, and OD was measured. There were 4 replicates of each pH solution. Graphs were drawn over the average results (standard deviation = 0.08).

Effect of temperature on growth

Clostridia media and thioglycolate broth with resazurine were used in this experiment. The media were dispensed into 100 ml serum bottles, purged with nitrogen and autoclaved at 121oC for 2 min. The pH was adjusted to 6.60 and 7.10 for both media. All the serums bottles were inoculated with 0.5 ml culture with a sterile needle and syringe and incubated in an anaerobic jar at 50, 55, 60, 65, 70, and 75 oC for 42 h .OD was measured and graphs were drawn over the average results of 4 replicates (standard deviation = 0.1) The temperature of the incubation oven was monitored with a calibrated digital thermometer.

Results

Growth in nutrients

The ability to utilize various substrates was tested and given as a function of optical density (OD) (Table 1). The OD of the growth showed that the highest growth occurred in the clostridial nutrient media with and without vitamins. The use of thioglycolate broth with vitamins resulted in a better growth than thioglycolate broth without vitamins. The glucose based medium with or without vitamins showed little or no growth. All the results were obtained after 42 h of incubation, the glucose medium when incubated for a period of 4 days longer showed only a minimal extra growth. These results suggest that both clostridial and thioglycolate can be a better substrate than glucose based medium for cultivation of Thermoanerobacter brockii subsp. lactiethylicus 9801T as they provide enough nourishment for the bacteria to survive with a reasonable growth rate. Thus, the two media were selected for evaluation of the optimum pH for growth. Figure showed the phase contrast micrograph of Thermoanerobacter brockii subsp. lactiethylicus 9801T. Table 1: The results of the different media used for growth based on optical ensity measurement Media Optical Density (650 nm) Glucose based medium with 0.2 ml trace elements and 0.2 ml vitamin solution 0.328 Glucose based medium with 0.2 ml trace elements ithout vitamins 0.156 Glucose based medium without trace elements and vitamin solution 0.051 Glucose based medium without trace elements and with 0.2 ml vitamin solution 0.013 Clostridial utrient medium without vitamins 1.344 Clostridial nutrient medium with 0.2 ml vitamins 1.270 Thioglycolate broth with resazurine without vitamins 0.904 Thioglycolate broth with resazurine ith 0.2 ml vitamins Figure 2: Phase-contrast micrograph of Thermoanerobacter brockii subsp. actiethylicus 9801T (x 40).

Effect of temperature on growth

As shown in Figure 4, growth was observed at a temperature range of 45-70^{o}C for the two substrates. The OD was highest at 55^{o}C for reinforced clostridia media; however for the thioglycolate the OD was slightly lower at 55^{o}C. When the two lines for the broths were statistically compared by running an F-test, both averages was found to be the same. It can be concluded that given a pH of 6.6 affords the best optimal temperature for this strain to be deduced in a substrate. Since the clostridial nutrient medium gives the best result for optimum temperature, further experiments conducted uses this substrate as growth media. It is time saving and affords the opportunity to concentrate entirely on a single substrate. Figure: Temperature optimum after 24 h incubation

Conclusion and Findings

Thermoanaerobacter brockii subsp. lactiethylicus 9801T has been investigated as a suitable candidate for microbial enhanced oil recovery. From this investigation, it can be concluded that

Thermoanaerobacter brockii subsp. lactiethylicus 9801T have a potential for possible utilization in microbial enhanced oil recovery. This is based on the ability of this strain to grow at an optimal temperature of 55^{o}C and production of metabolites that can be utilized for MEOR purposes. The strain was capable of producing organic acids that can modify rock properties evident in dissolution of chalk samples. Cells were also able to migrate through pore spaces of carbonate rock sample suggesting possibility of high mobility when injected for microbial enhanced oil recovery purpose. Degradation of the alkanes was also significant. It is evident that this strain offer useful metabolic products such as biosurfactants, biogas, biomass, in addition to bio-acids for enhancing oil recovery. These bio-products can contribute to different microbial systems which can tackle specific problems of oil recovery. However, the finding indicated that the range of salinity at which this strain could grow is low compared to saline content of many oil wells but it could provide an opportunity for further research in terms of its applications in microbial enhanced oil recovery.

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