DEVELOPMENT OF WATER GLASS MEDIUM FOR DETECTION OF MICROORGANISMS WITH CRUDE OIL BIODEGRADATION ACTIVITY

VÝVOJ MÉDIA PRO DETEKCI MIKROORGANISMŮ DEGRADUJÍCÍH ROPNÉ LÁTKY NA BÁZI VODNÍHO SKLA

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Abstract:

It was developed the water glass cultivation medium with the potential for the application in the monitoring of the effectivity of bioremediation technologies. The water glass medium is useful for the detection of the growth of microorganisms in crude oil environment and their crude oil degradation activity.

It was recognized that the quality of the gel and the detection of crude oil degradation are influenced by external parameters such as pH value, water glass concentration, and mode of heat treatment (temperature and time). The results obtained with application of water glass medium were compared with those obtained by using minimal medium that is usually used for crude oil degradation activity.

Keywords:

Microorganisms, degradation, crude oil products, solidifying agent, water glass

Introduction

Crude oil and oil products are very dangerous to the environment. They can damage all its parts - soil, water and air. Crude oil and its products can get in the environment by any manipulation with them - extraction, transportation, storage, processing, usage, and in the largest quantities in oil spills. Oil hydrocarbons can be biodegraded, so bioremediation technology can be used for removal of unwanted oil substances. The information about microorganisms inhabiting the contaminated environment and determination of their bioremediation potential is very important to the design the bioremediation strategies and their monitoring (Horáková, 2006).

Bioremediation activity is detected on minimal media. These media contain minimum of substances which can influence growth of microorganisms in order to determine if the microorganisms can utilize the substrate (contaminant). There are solid or aqueous media. The solid media are prepared as aqueous, in addition they contain solidifying agent. The most widely used solidifying agent in microbiology is agar.

The main reasons for its use are stability, high purity, non-toxic character and resistance to metabolism of microorganism during culture period. It is a polysaccharide isolated from marine red algae of the genus *Gelidium*, *Gracillaria* and *Pterocladia*. Nowadays, it is becoming difficult to obtain sufficient amount of algae, hence agar price rises. Therefore, it is appropriate to find an alternative solidifying agent. Desirable properties of solidifying agent are rigidity of medium in a large temperature range, resistance against splitting by microorganism's enzymes (Watson Apirion, 1976; Gardner, Jones, 1984). This alternatively solidifying agent can be the water glass. It is a colloidal solution of an alkaline silicate (sodium, potassium or lithium). Silicate is produced by melting the silicate sand with competent alkaline carbonate at a temperature about 1400 °C. Water glass is used as glue or as an ingredient in the degreasing, washing and cleaning agents and as a binder in the production of sand forms in foundries. The conversion of the gel occurs during evaporation of water, the change in pH to 5-6 or by adding electrolytes, organic and wit water-miscible solvents, the quarter bases, etc. (Kotlík, 1999).

The goal of the work was to creat medium in which the agar will be replaced by an alternative solidifying agent and which would exhibit similar properties as the medium solidified with agar.

Materials and methods

Applied microorganisms

Gordonia terrae G, *Variovorax* sp. V, yeast CYA were cultured aerobically at temperature 22 °C on all tested media.

Agar-based media

Minimal medium containing bacteriological agar (BSM) (Doláková, 2014), medium from demineralized water and bacteriological agar (Demi agar) were used as comparative media.

Preparation of water glass medium

The medium was prepared from demineralized water and water glass. The demineralized water was acidified by 25% (vol.) H_3PO_4. The pH of acidified solution with water glass was 7,6 ± 0,5. Various ratios of demineralized water and water glass (5:1, 7:1, 9:1, 14:1) with or without addition of saturated solution of Na_2SO_3 were tested. The filter paper with a solution of NaCl as desiccant was introduced into some of the petri dishes (Seki, 1973; Hausler, 1995).

The medium containing water glass was prepared in ratio 7:1. This medium was heated at 55 °C during 60, 120 min and at 100 °C during 10, 20, 30 min. Subsequently, medium was final dried at 20 °C. Further, the preparation and final drying at the temperature of 4-6 °C were tested.

Results

The medium was prepared from laboratory usage water glass and from water glass available in drugstore. There wasn't any difference among the results obtained from both of those media. The best volume ratio of demineralized water and water glass in the selection of concentration was 7:1. And it was used for the following experiments. The glass petri dishes were heat-treated at 100 °C and plastic petri dishes at 55 °C. After heat treatment at 100 °C the medium was cracked by bubbles that occurred during heating. At the temperature of 55 °C there was continuous release of water. At the temperature of 4-6 °C the created gel was solid and homogenous structure applicable for detection of crude oil degrading microorganisms.

There were used crude oil as a substrate and three microorganisms *Gordonia terrae* G, *Variovorax* sp. V and yeast CYA. The results obtained in the water glass medium were compared with results from BSM agar and Demi agar. Table 1 shows the average growth of microorganism on the water glass medium and other media. Different ways of substrate application were compared and each method was repeted two or three times. The water glass medium can replace commonly used minimal media. Both biodegradation activity and growth of microorganisms were observed. Compared to with BSM agar and Demi agar the biodegradation and growth occurs later and less expressive.

Tab. 1: Comparison of the growth of microorganisms on media containing crude oil

microorganism	water glass medium	BSM agar	Demi agar
G	3	3	3
V	1	2	2
CYA	2	2	2

Glossary: 1 … small microbial growth (less than 0,1 cm), 2 … medium microbial growth, 3 … large microbial growth (above 0,4 cm)

Discussion

For the preparation of water glass medium for detection of biodegradation was used direction for preparation of a medium containing water glass for psychrophilic microorganism in waters by Häusler (1995) where nutrient broth nutrients was used as a source of nutrients and ratio of water glass and acid solution was 1:9. During the cultivation, water glass medium releases water. To avoid this, Häusler added Na_2SO_3 to the acid solution during preparation and after mixing sample with medium the filter paper with desiccant ($CaCl_2$) was placed into the petri dish lid.

In our work, instead of nutrient broth we used acidified demineralized water. Because it was planned to use the medium for detection of degradation of contaminants in environment and contaminants are used as a source of nutrients, respectively source of carbon. The demineralized water was acidified

in order to final pH of ware glass medium was in neutral value and thus avoid the negative effect of pH on microbial growth.
For the heat treatment at 100 °C was used the method in the publication Bazylinsky and Rosenberg (1980) which is about the medium containing silica gel. As named in the publication of silica, structure of the medium was broken, which wasn't useful for the detection of bioremediation activity in contrast to silica medium.
That is the reason why low temperature was tested during the medium preparation (Seki, 1973). At this temperature the gel structure was similar to the consistency of agar and which could be used for the detection of microorganisms degrading crude oil.
The microbial growth and activity on the water glass medium are weaker in comparison to the results of obtained in BSM agar. In alignment with the previous measurements of monitoring microbial growth on medium from demineralized water with agar, this can be explained to the presence of agar which was served as a source of nutrients.

Conclusions
It was developed the water glass cultivation medium, the preparation is easier and less time consuming in comparison to the media containing silica. Also the post-manipulation is easier. There is eliminated post-contamination by absence of inserted filter paper with desiccant. The medium could have a good future in bioremediation technologies. The water glass fills the microbiological claims for inert substance; it is cheap and commercially available.

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References
Bazylinski, D.A., Rosenberg F.A 1980. Silica gel plates for culture of marine and nonmarine organisms. Appl. Environ. Microbiol. 39(4), pp. 934.
Doláková M. 2014. Metody detekce biodegradace ropných látek a nalezení potenciálních biodegradérů. Diplomová práce. VŠCHT Praha
Gardener S., Jones J.G. 1984. New solidifying agent for culture media which liquefies on cooling. J. Gen. Microbiol. 3, pp. 731 - 733.
Häusler J. 1995. Mikrobiologické kultivační metody kontroly jakosti vod: díl III. Stanovení mikrobiologických ukazatelů kap. 2.1.3 Stanovení psychrofilních bakterií v terénu na křemičitém kultivačním skle. Praha: Agrospoj.
Horáková D. 2006. Bioremediace. Brno: Masarykova univerzita.
Kotlík P. 1999. Stavební materiály historických objektů - materiály, koroze, sanace. Praha: Vysoká škola chemicko-technologická.
Seki H. 1973. Silica gel medium for enumeration of petroleumlytic microorganisms in the marine environment. Appl. Microbiol. 3, pp. 318 - 320.
Watson N., Apirion D. 1976. Substitute for agar in solid media for common usages in microbiology. Appl. Environ. Microbiol. 4, pp. 509 - 513.