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Original Research

The Ability of *Candida Maltosa* for Hydrocarbon and Emulsified Hydrocarbon Degradation

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Abstract

The yeast strain *Candida maltosa* EH 15 was used as a biological agent in the hydrocarbon and emulsified hydrocarbon biodegradation. Six different surface active compounds were used to emulsify hydrocarbons. Biodegradation degree and biomass quantity were determined daily over 7 days. The obtained results indicated the strong ability of *Candida maltosa* EH 15 for hydrocarbon biodegradation. The addition of the tested surfactants influenced hydrocarbon biodegradation; however, biodegradation effectiveness varies with the type and concentration of surfactant.

Keywords: biodegradation, *Candida maltosa*, hydrocarbon, rhamnolipides, saponin

Introduction

Contamination of soil and water is frequently caused by oil and oil related compounds, such as diesel oil. In recent years bioremediation has become one of the methods used in the restoration of oil-polluted sites. Oil spills not only devastate soil and aquatic ecosystems, but may also cause an alteration in microbial processes. Therefore, it is necessary to study the microbial degradation of crude oil as an environmentally friendly way of clearing up oil-polluted areas.

The first step in such studies is the isolation and identification of microorganisms from soil and water which are capable of crude oil degradation. Among many studies conducted on microbial biodegradation of oil-related contaminants, more than 80% are devoted to bacterial biodegradation. Bacteria are the most studied microorganisms and the participation of bacteria during hydrocarbon mineralization in soil has been studied by many researchers [1, 2]. However, it is not only bacteria that are capable of hydrocarbon biodegradation. Also, yeasts isolated from hydrocarbon contaminated sites display the ability to utilise oil-related compounds. According to many authors, bacteria have been described as being more efficient hydrocarbon degraders than yeast, or at least that bacteria are more commonly used as a test microorganism. However, there is information that yeasts are better hydrocarbon degraders than bacteria [3]. Many other investigators have reported the involvement of bacteria and yeast in crude oil biodegradation [4-10]. Moreover, yeasts and moulds are capable of utilizing a wide range of different carbon sources [11-13], though only a few studies have been carried out on the potential use of yeast on oil-contaminated sites [9]. The *Candida maltosa* strain is a very useful species by which one can investigate the physiology, biochemistry and molecular biology of such a hydrocarbon-assimilating microorganism. The species is found frequently in air, soil, and water, especially within sites enriched with hydrocarbons [11].

The growth of microorganisms on hydrocarbons is often accompanied by the emulsification of the insoluble carbon source in the culture medium. In most cases, this has been due to the production of extracellular emulsifying agents called biosurfactants [14]. Generally, surface--active compounds may influence hydrocarbon biodegradation by increasing the solubility of hydrocarbons or

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they may interfere with the cell walls of microorganisms. The aim of our research was to study the influence of six various surface agents (natural and synthetic) on the acceleration of a hydrocarbon model mixture biodegradation by *Candida maltosa* strain EH 15.

Experimental Procedures

Microorganism and Cultivation Conditions

The yeast strain *Candida maltosa* EH 15 used throughout this work was kindly supplied by Umweltforschungszentrum GmbH, Leipzig-Halle, Germany. The media used for the growth of yeast were sterilized in an autoclave (121°C, 0.1 MPa) for 20 minutes except for $FeSO_4 \cdot 7H_2O$, which was sterilized by filtration. The basic medium used throughout these studies consisted of (g l^{-1}): NH_4Cl, 3.0; KH_2PO_4, 0.70; $MgSO_4 \cdot 7H_2O$, 0.35; $FeSO_4 \cdot 7H_2O$, 0.035; $CuSO_4 \cdot 5H_2O$, 0.2; $MnSO_4 \cdot 5H_2O$, 0.2; $ZnCl_2$, 0.105; $CoSO_4 \cdot 7H_2O$, 0.025; H_3BO_3, 0.285. This medium was prepared according to Behrens [15]. Each flask was suplemented with $CaCO_3$, 0.5 g. A liquid culture was started by adding a loop full of cells from a standard agar plate in to a 250 ml Erlenmeyer flask containing a 50 ml medium. This cultivation was performed for 5 days, and 9 ml of this liquid culture was used for the inoculation of the final culture. Final experiments were performed in a 500 ml Erlenmeyer flask containing a 100 ml medium for 7 days. The flasks were incubated in a temperature-regulated chamber at 25°C and shaken at 120 rpm.

Biodegradation Studies

A model mixture of hydrocarbon (dodecane and hexadecane, 1:1 w/w) was used for estimating the influence of surfactants on biodegradation. Hydrocarbon concentration in the experiments was 3.75% (w/v). Experiments were performed in an oil/water emulsion. Each experiment was repeated five times, values for biomass and biodegradation are calculated as an average value out of five flasks with a precision of ± 4.3% for biodegradation and ± 0.03g l^{-1} for biomass. Biomass was determined by centrifugation at 8000 g for 10 min. Biomass was washed twice with 10 ml of acetone to remove alkanes. The pellet was dried at 105°C (24 h) and weighed. Hydrocarbon content was determined using an extraction of hydrocarbon by diethyl ether according to the standard method for oil gravimetric determination. The degree of biodegradation was calculated as $[1 - (X_o - X_1)/X_o]$ 100% [%], where X_o – initial amount of hydrocarbon, X_1 – amount of hydrocarbon after biodegradation.

Surfactants

The following surface active agents were used:

- lecithin extracted from soy beans , Sigma Aldrich,
- rhamnolipids, glycolipids produced by the bacteria Pseudomonas aeruginosa, Jeneil Biosurfactant Company, USA,
- saponin, glycoside, nonionic natural surfactants, Quillaja Bark, Sigma Aldrich,
- Lutensol GD 70 (polyglucosidases with C_8-C_{10} alkyl), BASF AG Ludwigshafen,
- Triton X 100 (polyethylene glycol tert-octylphenyl ether), Sigma Aldrich,
- Tween 20 (polyoxyethylene (20) – sorbitanemonolaureate), Sigma Aldrich.

All surfactants were used at 150, 300 and 600 mg l^{-1} concentrations.

Experiments were carried out over 7 days, whereas for kinetic studies samples were taken daily.

Results and Discussion

Our interest was drawn to the addition of surfactants and their influence on the degree of hydrocarbon biodegradation. Synthetic as well as natural surfactants were used throughout this study. The obtained results have proven that the *Candida maltosa* EH 15 strain displays excellent affiliation to hydrocarbon, which was expressed as a high biodegradation degree (57%).

The addition of exogenous surfactant led to a more efficient use of hydrocarbon by the tested strains. However, there was no simple correlation between the surfactant, its concentration and resulting biodegradation degree (Tables 1, 2). During kinetic studies of emulsified hydrocarbon biodegradation, no significant pattern or relation was observed.

Best results were obtained for saponin. Although Soeder [16] reported that despite an efficient solubilization, the bioavailability of PAH was not improved by quillaja saponin, in our experiments with this natural surfactant, biodegradation of alkanes fluctuated around 90% for both 150 and 300 mg/l concentrations. These results were more than 60% better in comparison to a system without surfactant. Therefore, one can assume that saponin did positively influence the bioavailability of the hydrocarbon mixture tested. However, under all conditions saponin never decreased the rate of hydrocarbon elimination. This was also observed by Soeder [16]. A subsequent increase in surfactant concentration did not cause a significant biodegradation rise, moreover for 600 mg/l saponin the biodegradation level was only 62%. This is caused by the antifungal and antiyeast activity of saponins. According to Sparg [17] the crude saponin mixture from Chenopodium quinoa can inhibit the growth of *Candida albicans* at 50 μg/ml, whereas the pure compounds show little or no activity. The critical micelar concentration (CMC) values for different saponins range from 1 μg/ml to > 1000 μg/ml. However, when testing for novel pharmacological compounds, CMC values of > 1000 μg/ml are generally too weak to be considered active and should be reported as inactive [17]. During our research quillaja saponin presented me-

Table 1. Influence of natural surfactants at different concentrations on hydrocarbon degradation by strain EH15 over 7 days.

Day of process	Hydrocarbon biodeg. [%]	Biodegradation by natural surfactants [%]								
		Concentration of rhamnolipid [mg/l]			Concentration of saponin [mg/l]			Concentration of lecithine [mg/l]		
	0.0	150	300	600	150	300	600	150	300	600
1	5.3	28.8	27.6	24.5	14.0	16.0	11.7	28.3	18.5	15.9
2	19.5	39.0	39.5	27.5	30.8	32.3	19.3	31.4	22.5	22.3
3	36.3	44.9	40.8	49.7	40.4	47.1	30.0	40.0	30.3	24.9
4	43.4	62.8	50.7	64.5	62.9	64.6	41.8	50.3	37.9	29.3
5	52.2	67.4	54.1	67.0	76.2	76.2	51.9	59.0	43.9	34.5
6	54.7	74.7	59.0	67.9	87.7	86.8	58.7	60.3	47.5	41.10
7	56.5	80.9	66.5	69.5	90.3	92.7	62.0	60.9	49.2	44.4

Table 2. Influence of synthetic surfactants at different concentrations on hydrocarbon degradation by strain EH15 over 7 days.

Day of process	Hydrocarbon biodeg. [%]	Biodegradation by natural surfactants [%]								
		Concentration of Lutensol GD 70 [mg/l]			Concentration of Triton X 100 [mg/l]			Concentration of Tween 20 [mg/l]		
	0.0	150	300	600	150	300	600	150	300	600
1	5.3	23.2	8.3	3.2	29.1	26.4	28.3	24.3	23.2	19.1
2	19.5	30.0	12.9	11.2	29.3	33.1	30.5	37.1	28.9	28.3
3	36.3	34.1	39.5	28.0	29.4	41.2	37.1	48.4	40.9	38.0
4	43.4	44.2	52.5	47.3	49.7	48.6	45.2	56.7	52.3	47.2
5	52.2	55.1	55.7	58.8	60.8	53.6	53.3	66.9	67.1	52.4
6	54.7	65.4	57.0	64.5	67.2	57.9	59.0	70.5	75.3	59.9
7	56.5	71.4	57.6	65.7	68.5	61.2	63.2	76.7	79.8	65.8

dium anti-yeast activity with growth-inhibitory effects above 600 μg/ml.

When rhamnolipids where used as an emulsifying agent, a similar tendency could be observed. Simultaneously, with an increase in surfactant concentration, a decrease in biodegradation was observed. The optimal concentration was 150 mg/l, with a biodegradation degree of 81%. Such results of the significant acceleration of the biodegradation of octadecane at low rhamnolipids concentrations were also observed by Churchill et al. [18]. At the concentration of 600 mg/l the rhamnolipids inhibited the mineralization of octadecane. Similar behaviour of this biosurfactant was observed during our research.

Lecithin was generally thought to be an inappropriate surface active agent. The addition of this compound had no impact on biodegradation in the case of the lowest concentration or performed as an inhibitor, lowering hydrocarbon usage (45%). This result is rather strange considering the general opinion about lecithin as a harmless compound [16].

The data provided by Laouar [19] has shown that growth of yeast in the presence of certain synthetic surfactants (eg. Triton X 100) increases the inherent permeability of the cell. Nevertheless, one must be aware that both natural and synthetic surfactants can change the permeability of the microbial cell. The multistep process of interaction between surfactant and yeast cell is incorporated into hydrocarbon uptake. Our result suggests the better performance of natural surface active agents. Although all active synthetic surface agents behaved as neutral or positive agents, none decreased biodegradation or caused lower biomass production; however, only Tween at 0.4% and 0.8% concentrations led to approximately 75% biodegradation. Figure 1 shows the real influence of all exogenously added surfactants, and is expressed as a coefficient. The coefficient is a simple biodegradation with one particular surfactant divided by biodegradation without any surfactants added. In order to compare the influence of added surfactants on biomass production and the biodegradation process, a simple biomass/biodegradation coefficient was introduced. It was

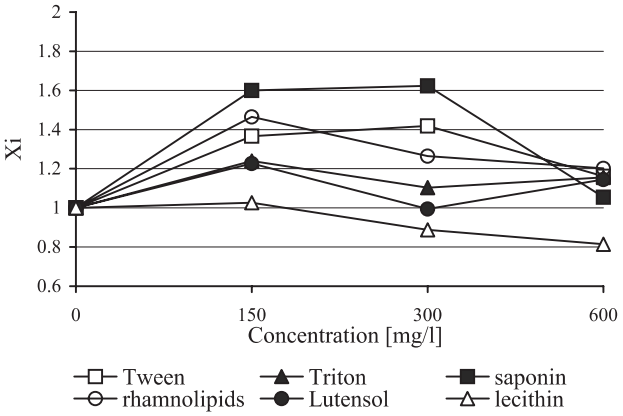

Fig. 1. Influence of surfactants on hydrocarbon biodegradation (*Candida maltosa*) expressed as the biodegradation of emulsified hydrocarbon : hydrocarbon biodegradation ratio, X_i - biodegradation of emulsified hydrocarbon/ hydrocarbon biodegradation.

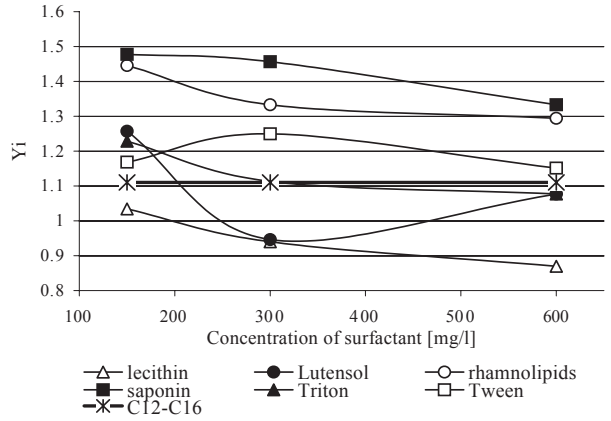

Fig. 2. Mutual relation between biomass, biodegradation and surfactant concentration, Y_i - Biomass/Biodegradation coefficient.

calculated as a ratio of biomass value [g] to biodegradation [%] multiplied by 100. For the pure degradation of hydrocarbon without any additional surfactant, the value of this coefficient was within 1.111. Data for all experiments (each biodegradation and biomass value was calculated as an average from five experiments) are presented in Figure 2. The best results were obtained for both saponin and rhamnolipids at 150 mg/l concentration. The obtained results clearly showed that the addition of surfactant could dramatically change the degree of degradation after 7 days incubation, in both positive and negative manner. Hence the choice of surfactants is crucial in order to achieve the best bioremediation results.

It has been demonstrated that hydrocarbons can be removed faster and biomass formation may also be manipulated by using appropriate surfactants. *Candida maltosa* used throughout this study is not known to produce any surfactant, thus the influence of exogenously added surface active compounds on the transport and translocation of the insoluble hydrocarbon across cell membranes could be observed.

Acknowledgements

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References

1. ATLAS R.M. Microbial degradation of petroleum hydrocarbons; an environmental perspective. Microbiol. Rev. **45**, 180, **1981**.
2. KIYOHARA H., NAGO K., YANA K. Rapid screen for bacteria degrading water-insoluble, solid hydrocarbons on agar plates. Appl. Environ. Microb. **43** (2), 454, **1982**.
3. IJAH U.J.J. Studies on relative capabilities of bacterial and yeast isolates from tropical soil in degradating crude oil. Waste Manage. **18**, 293, **1998**.
4. SONG H.G., PREDERSEN T.A., BARTHA R. Hydrocarbon mineralization in soil: Relative bacterial and fungal contribution. Soil Biol. Biochem. **28**, 109, **1986**.
5. FEDORAK P.M., SEMPLE K.M., WESTLAKE D.W.S. Oil degrading capabilities of yeasts and fungi isolated from coastal marine environments. Can. J. Microbiol. **30**, 565, **1984**.
6. OKPOKWASILI G.C., AMANCHUKWU Sc. 'Petroleum hydrocarbon degradation by *Candida* species. Environ. Int. **14**, 243, **1988**.
7. ANTAI S.P., MGBOMO E. Distribution of hydrocarbon utilizing bacteria in oil spill areas. Microbiol. Lett. **40**, 137, **1989**.
8. RAHMAN K.S.M., BANAT I.M., THAHIRA J., THAYUMANAVAN THA., LAKSHMANAPERUMALSAMY P. Towards efficient crude oil degradation by a mixed bacterial consortium. Bioresource Technol. **85**, 257, **2002**.
9. MARGESI R., SCHINNER F. Effect of temperature on oil degradation by psychrotrophic yeast in liquid culture and in soil. FEMS Microbiol. Ecol. **24**, 243, **1997**.
10. KATSIVELA E., MOORE E.R.B., KALOGERAKIS N. Biodegradation of Aliphatic and Aromatic Hydrocarbons: Specificity among Bacteria Isolated from Refinery Waste Sludge. Water Air Soil Poll. **3**, 103, **2003**.
11. SPENCER J.F.T., RAGOUT DE SPENCER, A.L., LALUCE C., Non-conventional yeast. Appl. Microbiol. Biotechnol. **58**, 147, **2002**.
12. ULFIG K., PŁAZA G. Keratinolytic fungi as indicators as hydrocarbon contamination and bioremediation progress in a petroleum refinery. Pol. J. Environ. Stud. **12** , 245, **2003**.
13. HOFMANN K.H., SCHAUER F. Utilization of phenol by hydrocarbon assimilating yeast. Anton. Leeuw. **54**, 179, **1988**.
14. PARRA J.L. Studies of biosurfactants obtained from olive oil. Tenside Surfact. Det. **27**, 302, **1990**.
15. BEHRENS U., WEIßBRODT E., LEHMANN W. Zur Kinetik der Citronensäurebildung bei Candida lipolytica. Z Allg Mikrobiol. **18,** 549, **1978**.

16. SOEDER C.J., PAPADEROS A., KLEESPIES M., KNEIFEL H., HAEGEL F.H., WEBB L. Influence of phytogenic surfactants (quillaya saponin and soya lecithi) on bio-elimination of phenanthrene and fluoranthene by three bacteria. Appl. Microbiol. Biotechnol. **44**, 65, **1996**.
17. SPARG S.G., LIGHT M.E., STADEN J. Biological activities and distribution of plant saponins. J. Ethnopharma. **94**, 219, **2004**.
18. CHURCHILL S.A., GRIFFIN R.A., JONES L.P., CHURCHILL P.F. Biodegradation Rate Enhancement of hydrocarbons by an oleophilic fertilizer and a rhamnolipid biosurfactant. J. Environ. Qual. **24**, 19, **1995**.
19. LAOUAR L., LOWE K.C., MULLIGAN B.J. Yeast responses to non-ionic surfactants. Enzyme. Microb. Technol. **18**, 433, **1996**.