

Efficient Removal of Unctuous Wastes from Wastewater

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Abstract—Nowadays, food industrial unctuous wastes represent a serious problem in industrial wastewater treatment processes. As a consequence of their hydrophobic characters, their emission results in dramatic environmental and economical effects. Unctuous materials, depositing in the pipelines, block the waste flow, furthermore reduce the efficacy of the wastewater treatment. However, bioremediation processes - using microorganisms to degrade these hazardous materials - can provide a solution for this problem. Several rhodococci are able to assimilate many hydrophobic materials converting them into biomass and CO₂. These bacteria use surfactants for making these hydrophobic materials suitable for aqueous bioconversion processes. Additionally, surfactant producing bacteria are capable to decompose many types of hydrocarbons present in diesel oil or dead oils, as well. Rhodococci also have monooxygenase enzymes capable to catalyze the oxidation of numerous hazardous hydrocarbons. Moreover, these strains might harbor lipase enzymes as well, which might help in the degradation of unctuous wastes, such as pig lard or poultry fat.

In this study, our aim was to demonstrate the qualitative ability of *Rhodococcus* sp. strain to degrade unctuous wastes released by food industry and domestic activity. The strain shows high similarity in its physiology to the *R. erythropolis* PR4 (NBRC 100887), but apparently of their surfactants are different. Nevertheless, this *Rhodococcus* sp. MK1 strain was able to use pig lard and poultry fat as sole carbon sources in minimal medium.

Index Terms—Bioremediation, rhodococci, unctuous wastes, surfactants.

I. INTRODUCTION

Since the last century, industrial emission of harmful materials is an extremely acute problem for humanity and Nature. Technologies with low or zero emission is of key importance to minimize the contamination of the ecosystem. However, vast amount of hazardous substances still gets out into the environment which must be made harmless. Bioremediation technologies using microorganisms to neutralize polluting materials are environmentally sound and economical tools.

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It is very hard to neutralize various oils and their derivatives (n-alkanes, aliphatic-, aromatic hydrocarbons) because of their physico-chemical properties. Fortunately, there are „oil eater” microbes capable for decomposition and assimilation of much type of hydrocarbons [1]-[5]. The bioremediation of diesel oil is a relatively simple process, because it contains mainly linear alkanes. During aerob biodegradation, microorganisms such as *Rhodococcus* sp. [5], [6] and *Pseudomonas* sp. [7] oxidize these compounds with their monooxygenases which is followed by a successive biochemical reaction set completing the conversion [8]-[11]. Most of the hydrocarbons are insoluble in water so unavailable for microorganisms [1], [2]. Some species, such as like pseudomonads, rhodococci, bacilli, synthesise surfactants to solubilize otherwise unaccessible organic carbon sources [12]. These microorganisms improve the bioavailability of such hydrophobic compounds by their surfactants [13]-[15]. These surfactants might be extracellular or bound to the membrane [12], [13].

Our model strain - *Rhodococcus* sp. MK1 strain - was isolated from a hydrocarbon polluted site and it was successfully proven that the bacterium could efficiently degrade industrial hydrocarbons, such as diesel oil and dead oil [16], [17]. This strain could tolerate low temperature and high salt concentrations therefore it might be applied in oil mineralization on the sites at the oil spills, mainly in marine areas.

However, in addition to the crude oil and its derivatives, it is also important to handle of the food industrial and municipal unctuous wastes in an environmentally sound way.

The aim of this work was to compare the unctuous-waste-degrading-ability of our *Rhodococcus* sp. strain. The *Rhodococcus* sp. MK1 had cell wall-bound surfactant, in spite of other *Rhodococcus* strain, e.g. the *Rhodococcus erythropolis* PR4 released its extracellular surfactant into the extracellular space. The strain PR4 produces fatty acid containing extracellular polysaccharide which can emulsify the hydrocarbons [18], [19].

Although oil and its derivative and animal fats have distinct chemical characteristics, these strains might be versatile and might synthesise lipase enzymes, as well, which can catalyse the conversion of the unctuous wastes such as pig lard, and poultry fat [20].

Thus, the goal of this work was to get a qualitative picture about our isolate whether it could utilize hydrophobic organic carbons, such as pig lard and poultry fat as sole carbon sources. Pig lard and poultry fat were used as sole carbon sources in minimal medium. The biodegradation processes were followed by measuring the respiration activity and CO₂ emission of the cells.

II. MATERIALS AND METHODS

A. Microorganisms

Rhodococcus sp. MK1 was isolated from hydrocarbon polluted site at Mohács, Hungary [16].

B. Materials Used

Carbon sources: poultry fat, pig lard (domestic waste), diesel- and dead oil (came from a farmland) were sterilized at 100 °C for 1 hour before use.

Luria-Bertani medium (LB): 1L medium contains 5g yeast extract, 10g Trypton and 10g NaCl (20 g/L agar for agar plates) This medium was used to maintain the rhodococci strains.

Minimal salt medium (MSM) contains 0,68 g/l KH_2PO_4 , 0,87 g/L K_2HPO_4 , 0,58 g/L NaCl, 0,125 g/L $\text{MgSO}_4 \times 7\text{H}_2\text{O}$, 0,044 g/L $\text{CaCl}_2 \times 2\text{H}_2\text{O}$, 0,0012 g/L NH_4NO_3 , 0,014 g/L FeSO_4 complexed with EDTA, 2 ml of trace element solution (pH=6,8).

Trace element solution: 0,1g/L $\text{ZnSO}_4 \times 7\text{H}_2\text{O}$, 0,03g/L $\text{MnCl}_2 \times 7\text{H}_2\text{O}$, 0,3g/L H_3BO_4 , 0,2g/L $\text{CoCl}_2 \times 6\text{H}_2\text{O}$, 0,01g/L $\text{CuCl}_2 \times 2\text{H}_2\text{O}$, 0,02g/L $\text{NiCl}_2 \times 6\text{H}_2\text{O}$, 0,03g/L $\text{NaMoO}_4 \times 6\text{H}_2\text{O}$

Chemicals: All materials were purchased from standard commercial suppliers (Reanal, Spektrum 3D, Merck, Sigma).

C. Bacterial Growth Conditions

Starter cultures were grown in 30 ml LB medium at 24 °C shaking with 150 rpm for one day. The cells were harvested by centrifugation (13.000 rpm for 10 min, 4 °C), the pellets were washed twice with 5 ml of sterile MSM to remove all organic matters. Then, the cells were suspended in MSM adjusting, the optical density of the cell suspension to $\text{OD}_{600}=1.0$. This suspension was used as an inoculum for the experiments at 1% v v⁻¹. The MSM was supplemented with 1% v v⁻¹ diesel oil, dead oil, pig lard or poultry fat as the sole carbon source. The cultures were incubated in gas tight vials, and were shaken at 150 rpm at 24 °C for a week.

D. Gas Chromatography (GC)

The oxygen content of the headspace was measured by Agilent 6890 gas chromatograph equipped with a thermal conductivity detector (TCD) and a HP-MOLESIEVE column (30m x 0,53mm i.d. x 0,25µm). The injector was kept at 150 °C while the oven temperature was adjusted to 60 °C. The injector was in splitless mode and nitrogen was used as carrier gas.

For carbon dioxide measurements, Shimadzu 2010 gas chromatograph equipped with a TCD and HP-PlotQ column (30m x 0,53 i.d. x 0,25µm) was used. The temperatures of the injector and oven were 200 °C and 90 °C, respectively. Samples of 50µl were injected via a split injection port at a split ratio of 0, 5:1. Carrier gas was nitrogen at a flow rate of 63.8 mL/min.

III. RESULTS

A. *Rhodococcus* sp. MK1 Strain Consumes both Diesel and Dead Oil

During aerobic biodegradation, the cells use O₂ for

oxidation of the substrates and mainly biomass and CO₂ will be formed. Therefore, following the respiratory activity and CO₂ production of cells growing on unctuous substrates as sole carbon source are good tools to monitor the bioremediation processes.

Both diesel and dead oil seemed to be utilized by *Rhodococcus* sp. MK1 as sole carbon sources with different efficiencies. The cells were grown on diesel oil consumed all oxygen till the day 7, however, cells cultivated on dead oil used only about 50% of the available oxygen (Fig. 1). Carbon dioxide production was also 50% larger within this period for samples using diesel oil as compared to those propagated on dead oil (Fig. 1).

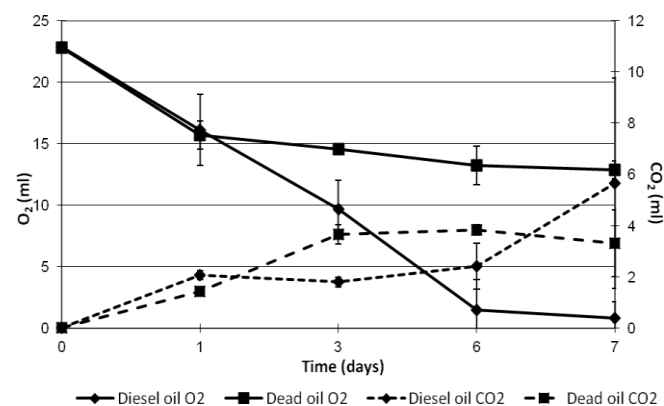


Fig. 1. Respiration activity and CO₂ production of *Rhodococcus* sp. MK1 grown on diesel oil and dead oil as carbon sources.

B. Our Isolate Could Efficiently Utilize Pig-, Poultry Fat, Lard and Cooking Oil

The bacterium could also decompose both lard carbon source, but the efficacies were slightly varied with the substrates (Fig. 2). Cell respiration was more intense on poultry fat than on pig lard. When poultry fat was the sole carbon source the cells consumed all of the available oxygen in 6 days, while in the case of lard, residual some oxygen could be detected after 7 days. Analysis of the CO₂ production led to similar conclusions. Cells grown on poultry fat released more CO₂ within shorter time as compared to the lard grown samples.

It is remarkable that - although no extreme differences could be observed in oxygen consumption during growth on lard, poultry fat (Fig. 2).

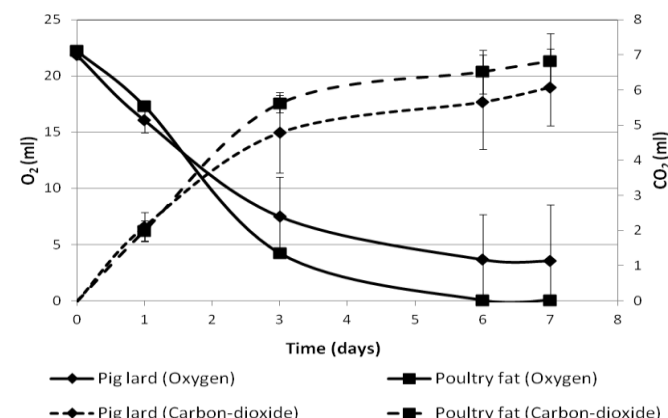


Fig. 2. Oxygen demand and CO₂ production of *Rhodococcus* sp. MK1 grown on pig lard, poultry fat.

IV. CONCLUSION

A. *Rhodococcus*

Strain apparently suitable for bioconversion of various kinds of hydrophobic carbon sources was isolated and its bioremediation capacity was evaluated.

The bioconversion processes were followed by indirect methods, carbon dioxide emission and respiration/oxygen consumption.

In the case of unctuous substrates, a quite fast adaptation was observed with our isolate names ad *Rhodococcus sp.* MK1.

In addition to diesel oil and its derivatives, these strains could efficiently decompose pig lard, poultry fat as single carbon and energy source.

Oil and its derivatives and animal fats have distinct chemical properties, consequently their catabolisms require alternative metabolic pathways, distinct enzymatic activities. However, the isolated strain was able to utilize both types of substrate indicating the presence of many versatile and biotechnologically beneficial metabolic routes in this microorganism.

This bacterium is truly promising waste cleaner both in environmental, food industrial applications and in housekeepings.

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