# BIODEGRADATION OF UNCTUOUS WASTES OF FOOD INDUSTRY

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## ABSTRACT

Nowadays, 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 for removal toxic compounds.

It is a well-known fact that several *Rhodococcus* sp. can degrade a wide range of hazardous chemicals, such as aliphatic and aromatic hydrocarbons. In our laboratory, a *Rhodococcus* sp. was isolated from hydrocarbon polluted sites and it was successfully proven that the bacterium could efficiently degrade industrial hydrocarbons such as diesel oil and dead oil. The strain could tolerate low temperature and certain salt concentrations therefore it might be applied in oil mineralization after marine catastrophes.

In this study, our aim was to test the ability of this strain to degrade food industrial and municipal waste.

Lard, pig and poultry fat and cooking oil were used as sole carbon sources in minimal medium. The substrate utilization was demonstrated indirectly by measuring the respiration activity and $CO_2$ production of the *Rhodococcus* sp. The strain could grow even at 10 g/l of hydrocarbon concentration, it consumed the available oxygen and released remarkable amount of carbon dioxide within a week. These facts make this strain a promising waste cleaner both in food industrial applications and housekeeping.

## 1. INTRODUCTION

Nowadays, a major environmental problem is the emission of hazardous materials by industry, agriculture and other human activities. 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 many type of hydrocarbons [1]. The bioremediation of diesel oil is a relatively simple process, because it contains mainly linear alkanes. During aerob biodegradation, microorganisms such as *Rhodococcus* sp. [2] and *Pseudomonas* sp. oxidize these compounds with their monooxygenases which is followed by a successive biochemical reaction set completing the degradation. These microorganisms improve the bioavailability of such hydrophobic compounds by their surfactants [2, 3].

A *Rhodococcus* sp. was isolated from a hydrocarbon polluted site. This strain was able to grow on diesel and dead oil as carbon sources in laboratory conditions.
However, in addition to crude oil and its derivatives, it is also important to neutralize food industrial and municipal unctuous wastes in an environmentally sound way. Thus, the goal of this project was to establish whether the strain could utilize organic carbons, such as pig-, poultry fat, lard and cooking oil, as sole carbon sources. The biodegradation processes were followed by measuring the respiration activity and $CO_2$ emission of the cells.

## 2. MATERIALS AND METHODS

### *2.1. Microorganism*

*Rhodococcus* sp. was isolated by us from hydrocarbon polluted site at Mohács, Hungary.

### *2.2. Materials used*

Carbon sources: pig-, poultry fat, lard, cooking oil, diesel and dead oil were sterilized at 100 °C for 1 hour before .
Luria-Bertani medium (LB): 1L medium contains 5g yeast extract, 10g Trypton and 10g NaCl (20 g/L agar for agar plates)
Minimal salt medium (MSM) containing 0,68 g/l $KH_2PO_4$, 0,87 g/L $K_2HPO_4$, 0,58 g/L NaCl, 0,125 g/L $MgSO_4 \times 7H_2O$, 0,044 g/L $CaCl_2 \times 2H_2O$, 0,0012 g/L $NH_4NO_3$, 0,014 g/L $FeSO_4$ comlexed with EDTA, 2 ml of trace element solution (pH=6,8).
Trace element solution: 0,1g/L $ZnSO_4 \times 7H_2O$, 0,03g/L $MnCl_2 \times 7H_2O$, 0,3g/L $H_3BO_4$, 0,2g/L $CoCl_2 \times 6H_2O$, 0,01g/L $CuCl_2 \times 2H_2O$, 0,02g/L $NiCl_2 \times 6H_2O$, 0,03g/L $NaMoO_4 \times 6H_2O$

### *2.3. Bacterial growth conditions*

Starter culture was grown in LB, the cells were collected by centrifugation then suspended in 25 ml MSM containing 1% (v/v) of the carbon sources. The flasks were shaked at150 rpm at room temperature. The control flasks did not contain cells.

### *2.4. 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 the carbon dioxide measurements, Shimadzu 2010 gas chromatograph was used, equipped with a TCD and HP-PlotQ column (30m x 0,53 i.d. x 0,25μm). The temperature of the injector and oven was 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.

## 3. RESULTS

### *3.1.* Rhodococcus *sp. consumes both diesel and dead oil*

During aerobic biodegradation, the cells use $O_2$ for oxidation of the substrates and mainly biomass and $CO_2$ will be formed. Therefore, following the respiratory activity and $CO_2$ production of cells growing on unctuous substrates as sole carbon source are good tools to monitor the cellular activities, the bioremediation processes.
The diesel and dead oil were utilized by *Rhodococcus sp.* as sole carbon sources with different efficiencies. The cells were grown on diesel oil consumed all oxygen till the 7. day, 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).

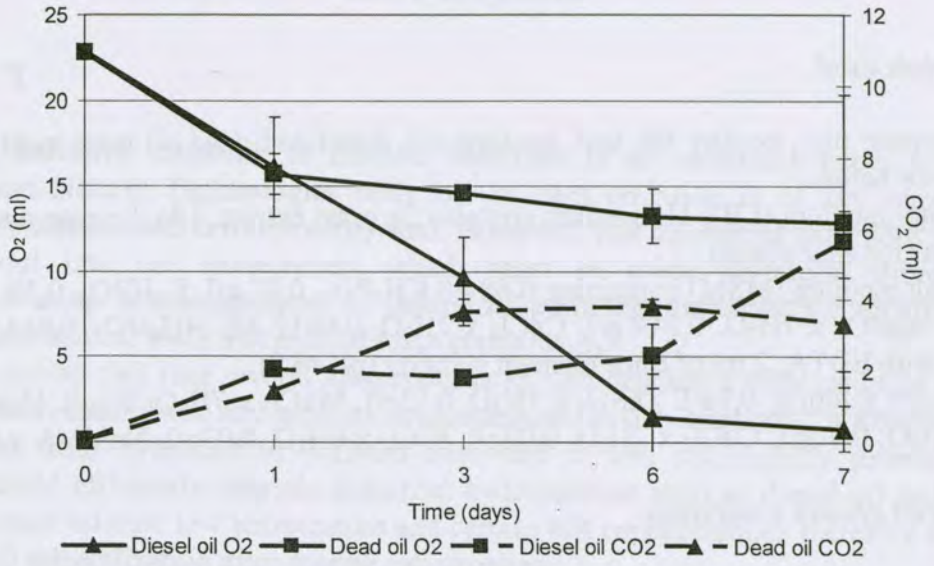


**Fig 1. Respiration activity and $CO_2$ production of *Rhodococcus* sp. grown on diesel oil and dead oil as carbon sources**

### *3.2. Our isolate could efficiently utilize pig-, poultry fat, lard and cooking oil*

The bacterium could also decompose both leaf-lard and lard carbon sources, but the efficiencies were slightly varied with the substrates (Fig. 2). Cell respiration was more intense on leaf-lard than on lard. When leaf-lard was the sole carbon source the cells consumed all of the available oxygen in 3 days, while in the case of lard, residual some oxygen could be detected after 7 days. Analyzing the $CO_2$ production led to similar conclusions. Cells grown on leaf-lard released more $CO_2$ within shorter time as compared to the lard grown samples.

It is remarkable that - although no serious differences could be observed in oxygen consumption during growth on leaf-lard, poultry fat or grease - the carbon dioxide emission was significantly less in the cultures cultivated on poultry relative to the other samples (Fig. 2).

The cooking oil is also an unctuous substrate of special physical and chemical properties. However, our *Rhodococcus* strain was able to grow well on cooking oil, as well. The cells

could be adopted rapidly to cooking oil floating on water surface. This degradation of the substrate was clearly confirmed by the persistent decreasing of the oxygen concentration and carbon dioxide release into the headspace of samples. Oxygen was completely taken up after 5-6 days and – accordingly – most of the $CO_2$ was produced till the 6th day (data not shown).

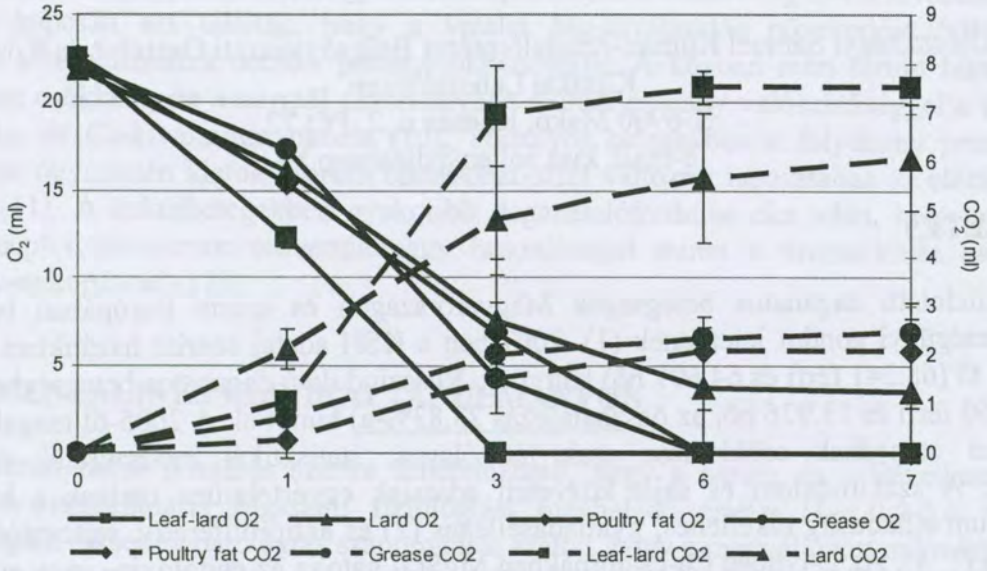


**Fig. 2. Oxygen demand and $CO_2$ production of *Rhodococcus* sp. grown on leaf-lard, lard, poultry fat and grease.**

## CONCLUSION

- Our *Rhodococcus* sp. isolate could be easily adopted to the unctuous carbon sources studied.
- In addition to diesel oil and its derivatives, this strain could efficiently decompose leaf-lard, poultry grease, lard, poultry fat and cooking oil as single carbon and energy source.
- Our microbe is a truly promising waste cleaner both in environmental, food industrial applications and in housekeeping.

## REFERENCES

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