# USING OF BIODAC (ABSORBENT GRANULES PRODUCED FROM PAPER INDUSTRY RESIDUES) AS CARRIER TO MICROORGANISMS FOR SOIL INOCULATION

**A. D. Ilyina, S. J. A. Villarreal, R. E. Rivera, G. Y. Garza, and M. J. Rodríguez**

**From recycling/de-inking pulp residues of paper industry, ThermoFiberGen Inc. (Bedford, Massachusetts) makes absorbent granules currently marketed under the trade name BIODAC. In this work it was demonstrated the possibility to use the granules of BIODAC 12/20 as the carrier to microorganisms that can be useful for bioremediation applications. As studying models the six different types of microorganisms (bacteria and fungi) isolated from petroleum hydrocarbon-contaminated soils and their consortium, were used. It was demonstrated that BIODAC did not inhibit the microbial growth in the liquid culture media as well as in the solid phase of agar-agar. The microorganisms were alive after desiccation with granules and their storage during 2 months at least. The major number of CFU (approximately at 6 times) was detected in the petroleum hydrocarbon-contaminated soil inoculated with preparation of consortium-BIODAC as compared with the soil inoculated with the same quantity of consortium only. The presence of BIODAC affected the soil capacity to retain the moisture. It was observed the buffer property of this product and that BIODAC using (at reasonable limits) did not affect the growth of plants (canary grass and kidney bean). Therefore, it may be applied as carrier to microbial inoculation of soils.**

## Introduction

An increase in the use and production of recycled paper in recent years leads paper companies to substantial increase of pulp residue. It results from the pulp de-inking, filtering and cleaning process, in which approximately 25% of the recycled fiber entering a pulp mill is lost to the waste stream, and must be disposed of at a substantial cost. Expense of the North American pulp and paper industry was estimated as $ 900 million to treat and dispose of approximately 9 million tons of pulp residue in the beginning years of the last decennium of twentieth century. The worldwide expenditures to treat and dispose of pulp residue in that time were approximately $ 2.5 billion. So, advances in science and technology since the industrial revolution have increasingly enabled humans to exploit natural resources. However, it has generated unprecedented disturbances in global elemental cycles [1]. The relatively sudden introduction of xenobiotic chemicals, or massive relocation of natural materials to different environmental compartments, can often overwhelm the self-cleaning capacity of recipient ecosystems and thus result in the accumulation of pollutants to problematic or even harmful levels [2]. In addition to minimizing the impact of future incidents by controlling contaminant input, pollutant decay must be accelerated to remedy existing problems.

TERMO FIBERGEN INC. (Bedford, Massachusetts) aims at ultimate utilization of pulp residue from recycling/de-inking paper mills. From pulp residue, THERMO FIBERGEN makes absorbent granules (currently marketed under the trade name BIODAC) as a carrier to deliver chemicals, for agricultural, lawn and garden, and other needs, such as oil and grease absorbents. The BIODAC is a cellulose complex consisting of paper fiber (47–53%), kaolin clay (28–34%), calcium carbonate (14–20%) and titanium dioxide (no more than 1%). Typically gray BIODAC granules with musty odor are currently available in 12/20 mesh, 16/30 mesh and 20/50 mesh. The THERMO FIBERGEN's mobile plant (a prototype of larger plants) recovers and cleans long cellulose fibers for their return to pulp mills, and the FIBERGEN's technology processes the remaining recoverable components of pulp residue, such as short cellulose fibers not usable for papermaking, lignin, hemicelluloses, and minerals (e. g., calcium carbonate), into value-added products. For this, THERMO FIBERGEN uses chemical processes, enzymatic reactions and microbial transformations.

The primary objective of this study is to examine experimentally the possibility to use BIODAC 12/20 (the numbers indicate the type of this vale-added products obtained from pulp residue of pulp and paper industry and differed from other type of BIODAC in size of granules) as the carrier to microorganisms that can be useful for bioremediation applications. In this communication we report on:

(i) description of some properties of BIODAC useful to be applied as carrier;

(ii) description of effect of BIODAC on the microbial growth in the liquid culture media as well as in the solid phase of agar-agar and petroleum hydrocarbon-contaminated soil applying the six different types of microorganisms (bacteria and fungi) previously isolated from contaminated soils and their consortium as studying models;

Departamento de Biotecnología, Facultad de Ciencias Químicas de la Universidad Autónoma de Coahuila. Blvd. V. Carranza and Ing. J. Cardenas V., Col. Republica, A.P. 935, C.P. 25000, Saltillo, Mexico. Fax: (52-84)-15 95 34, E-mail: ailina@alpha1.sal.uadec.mx.

(iii) the results of study of some possible side effects of BIODAC application (effect on soil capacity to retain the moisture and its influence on the growth of plants).

## Materials and Methods

All reagents applied in the present work were purchased from Sigma Chemical Company (USA).

Previously, the total microflora CFU of BIODAC was counted from appropriate dilutions by means of common microbiological technique [3] and some properties of this material were defined. To evaluate the real density the experiment with displacement of water was carried out [4]. The apparent density was detected by dividing the weight to volume. The porosity was calculated resting from 100% the division (multiplying for 100) between apparent and real densities [4]. Moreover, titration of the mixture of BIODAC (1 g) and bidistilled water (20 ml, pH 7) with 0.1 N NaOH and 0.1 N HCl was realized. The values of capacity to absorb oil and water were approximated by visual observation of saturation of 1 g of BIODAC after addition of the aliquots (0.01 ml) of these substances.

The microorganisms applied in the present work as model to study the effects of BIODAC on the microbial growth were isolated from six samples of petroleum hydrocarbon-contaminated soil. The collection of soil was realized from different sites of South of Mexico by COMIMSA (Coorporacin Mexicana de Investigacin en Materiales, S.A. de C.V.). The common microbiological techniques [3] were applied using the suspension of homogenized soil in solution (peptone of casein 1 g/L and NaCl 8.5 g/L of water) plated in duplicate on petri dishes containing nutrient agar, Sabourauds agar and agar-petroleum. The last contained petroleum (5 ml of Maya crude on 1 L of agar) as unique source of carbon. After incubation at 37 °C and ambient temperature (23 °C) for no more than 21 days the microscopic observation of colonies was effectuated for each medium and sample of soil. Six isolated colonies were selected because they were detected in each sample of soil. The identification of microorganisms was performed by means of biochemical essays commonly used in microbiological practice [3].

The effect of BIODAC on microbial growth in liquid medium was evaluated by comparison of the total microflora CFU detected from appropriate dilutions of suspensions of each type of microorganisms and their consortium incubated at 37 °C for 48 h in the presence of 2.5% and without (control essay) BIODAC 12/20. Then the granules were separated from the culture medium and desiccated at 39 °C in vacuum. After 2 months of storage at ambient temperature (23 °C in average) the viability of the microorganisms was demonstrated by means of common microbial technique using the petri dishes containing nutrient agar [3].

The effect of BIODAC 12/20 on microbial growth in solid phase was evaluated after incubation of microorganisms at 37 °C for 24 h and 96 h, respectively for bacterias and fungis, by means of visually comparison of cultures in petri dishes containing nutrient agar without (control) or with 0.5 g of BIODAC.

The experiments simulated the using of BIODAC12/20 as carrier of microorganisms on soil inoculation, were carried out applying the desiccated preparation of consortium-BIODAC (containing $1.43 \times 10^{11}$ CFU) and the suspension of microorganisms in the culture medium ($1.44 \times 10^{11}$ CFU). The soil was contaminated with petroleum adding 360 ml to 6 kg of soil. Three parts of petroleum-contained soil weighting 1.8 kg each, were introduced in similar plant pots and exposed to ambient condition. The total microflora CFU [3] was detected and the inoculation of two soil samples from three was performed. To inoculate the soil samples the 22 g of desiccate preparation of consortium-BIODAC (containing $1.43 \times 10^{11}$ CFU) and 60 ml of the suspension of microorganisms in the culture medium ($1.44 \times 10^{11}$ CFU) were added in the first and second plant pot, respectively. The third plant pot was used without inoculation to evaluate the microbial growth of resident microflora under the same conditions of experiment. The total microflora CFU was counted from appropriate dilutions by means of common microbiological technique after each 10 days.

Moreover, in the present work the possible side effects of BIODAC application (effect on soil capacity to retain the moisture and its influence on the growth of plants) were evaluated. Soil water retention (drainage) was determined from the loss of weight of samples (5 g) of acid, neuter or alkaline soils with 1 g of BIODAC 12/20 or without it (as control) drying the initially saturated by 7 ml of water soil sample in incubator at 50 °C.

The experiment with plants was carried out using 85 $cm^3$ of acid, neuter or alkaline soil with 5 g of BIODAC or without it (in control essays) and 5 g canary grass and 2.5 g of kidney bean seeds. The plant pots were maintained under laboratory conditions during 12 days adding the 30 ml of water initially and after each 48 h. In each case the time of seed germination and the height of plant and weight of produced biomass were detected. The samples of soil were proportionate by Universidad Autónoma Agraria Antonio Narro (Saltillo, Mexico).

## Results and Discussion

Bioremediation, a process that exploits the catalytic abilities of living organisms to enhance the rate or extent of pollutant destruction, is an important tool in attempts to mitigate environmental contamination [5, 6]. Bioremediation achieves contaminant decomposition or immobilization by exploiting the existing metabolic potential in microorganisms with novel catabolic functions derived through selection, or by the introduction of genes encoding such functions. The effectiveness of bioremediation is often a function of the extent to which a microbial population or consortium can be enriched and maintained in environment. When few or no indigenous degradative microorganisms exist in a contaminated area and practically does not allow time for the natural enrichment of a suitable population, inoculation may be a realistic option. Inoculation may sometimes shorten the acclimation period prior to the onset of biodegradation. The same ecological principles that influence biodegradation in general will also govern the effec-

tiveness of inocula, regardless of whether they are natural isolated or genetically engineered microorganisms [7].

We supported that the effectiveness of inoculation may be increase if the microorganisms are employed by means of absorbent granules of BIODAC as carrier. Initially, it was observed that BIODAC 12/20 granules contained amount of microorganisms (250 000 CFU/g). It may be considerate as indication that the BIODAC is not toxic for some type of microorganisms. The previous sterilization of granules is necessary and was carried out every time when they were applied in the experiments with microorganisms.

Moreover, previous experiments to define some properties of BIODAC 12/20, were carried out. The value of porosity of the granules was calculated as 65% basing on the data of real density ($1.93 \pm 0.53$ g/cm$^3$) and apparent density ($0.63 \pm 0.15$ g/cm$^3$) determined as described above. It was observed that BIODAC 12/20 absorbers hydrophilic and hydrophobic liquid, for example water (approximately 1.15 ml/g) and oil (approximately 1.00 ml/g), because of great cellulose content. This great value of porosity and capacity to absorber the hydrophobic and hydrophilic liquids may be useful for BIODAC application as carrier for microorganisms for bioremidiation objectives. These properties are important for distribution of microorganisms in the granules and absorption of substrates limiting the growth of microbial population and can be hydrophilic or hydrophobic. The mixture of BIODAC and bidistilled water demonstrated pH 8 and maintained pH at 8.4 after addition of 1.5 ml of 0.1 N NaOH as well as at 7.5 after addition of 1.5 ml of 0.1 N HCl at least that indicates on the buffer property of this product.

As studying models the six different types of microorganisms (bacteria and fungi) isolated previously from petroleum hydrocarbon-contaminated soils and their consortium, were used. They were: *Enterobacter sakasaki, Serratia marcesens, Aspergillus sp., Bacillus sp., Streptococcus sp., Mucor sp.* The results of Table 1 demonstrate that BIODAC does not inhibit the microbial growth neither in the liquid culture media no in the solid phase of agar and in some cases in the presence of this product the faster growth of microorganisms was observed. It is probably that the BIODAC favors to microbial growth due to its buffer property. Moreover, in the present study was observed that the microorganisms were alive after their desiccation with granules and after their storage during 2 months at least.

The experiments simulated the applying of BIODAC 12/20 as carrier of microorganisms on soil inoculation were carried out using the desiccate preparation of consortium-BIODAC and the suspension of microorganisms in the culture medium. The major number of CFU was detected in the petroleum hydrocarbon-contaminated soil inoculated with preparation of consortium-BIODAC (approximately at 6 times) as compared to the soil inoculated with the same quantity of consortium only (Table 2).

Microorganisms survive in habitat because they are metabolically able to exploit its resources and occupy a suitable niche [8]. Contaminants are often potential energy sources for microorganisms, yet the resident microflora may be incapable of exploiting such compounds. Under such

Table 1

**Evaluation of effect of BIODAC 12/20 on the microbial growth on in the liquid culture media and solid phase of agar-agar**

| Microorganism | Culture in liquid, CFU/ml | | Observation in solid phase of agar-agar |
|---|---|---|---|
| | With BIODAC 12/20 | Control (without BIODAC) | |
| *Enterobacter sakasaki* | $8.5 \times 10^{13}$ | $2.1 \times 10^{11}$ | Inhibition was not observed |
| *Aspergillus sp.* | $> 1 \times 10^{12}$ | $> 1 \times 10^{11}$ | Inhibition was not observed<br>Number of colonies was major in the zone with BIODAC |
| *Bacillus sp.* | $8.2 \times 10^{13}$ | $7.4 \times 10^{12}$ | Inhibition was not observed |
| *Streptococcus sp.* | $1.2 \times 10^{14}$ | $8.6 \times 10^{13}$ | Inhibition was not observed |
| *Mucor sp.* | $> 1 \times 10^{11}$ | $> 1 \times 10^{11}$ | Inhibition was not observed<br>Number of colonies was major in the zone with BIODAC |
| *Serratia marcesens* | $2.3 \times 10^{13}$ | $9.7 \times 10^{10}$ | Inhibition was not observed<br>Number of colonies was major in the zone with BIODAC |
| Consortium | $1.8 \times 10^{14}$ | $2.3 \times 10^{12}$ | Inhibition was not observed |

Table 2

**Number of microorganisms (CFU/g) in the petroleum hydrocarbon-contaminated soil inoculated with preparation of consortium-BIODAC, the suspension of microorganisms in the culture medium and without inoculation**

| Days | Without inoculation, CFU/ g | Consortium-BIODAC, CFU/g | Suspension of microorganisms, CFU/g |
|---|---|---|---|
| 3 | $2.23 \times 10^{10}$ | $2.24 \times 10^{10}$ | $2.43 \times 10^{10}$ |
| 13 | $2.64 \times 10^{10}$ | $4.48 \times 10^{10}$ | $5.8 \times 10^{10}$ |
| 23 | $3.79 \times 10^{10}$ | $192.00 \times 10^{10}$ | $299.00 \times 10^{10}$ |
| 33 | $10.30 \times 10^{10}$ | $1700.00 \times 10^{10}$ | $15.20 \times 10^{10}$ |
| 43 | $6.10 \times 10^{10}$ | $28.5 \times 10^{10}$ | $21.8 \times 10^{10}$ |

conditions, nonindigenous organisms able to use the pollutant chemical can establish themselves more readily. Since the survival of an inoculant is linked to the availability of the contaminant, such organisms eventually die back after the bioremediation has been accomplished. While it has been show to occur in some cases, some inocula have a tendency to remain at low levels, even without the selection pressure imposed by the contaminant. Whether this long-term survival of inoculants reflects a threshold population- density below which competition is less severe, or some unidentified starvation - survival mechanism, is still unknown [9].

The success of inoculation depends on the degradative capacity and the environmental competence of the inoculant. Many factors influence the function and survival of inoculants in the environment. These include abiotic factors such as the pH, temperature, redox potential and the availability of nutrients and water [3, 5, 10]. Similarly, biotic factors such as micorbial competition, amensalism and predation can limit the success of inoculants. Options for increasing the chances for successful inoculation include applying the inoculant in sufficient amounts, delivering it in suitable formulations, and creating a niche for the desired organism by simultaneously introducing a selective substrate [3]. The results obtained in the present work may be considerate as demonstration of an advantage of BIODAC application for contaminated soil inoculation.

Moreover, in this work we pay attention to the possible side effects that may be provoked by BIODAC application on soil. It was demonstrated that the presence of BIODAC affected the soil capacity to retain the moisture: it was more than 30% without product as compared to the soil with BIODAC. It is probably that this effect may be explained by the great porosity of BIODAC that simplifies the water evaporation. It may be useful to change the texture of some type of soils but indicate the necessity to take precaution applying the BIODAC as carrier in the dry climatic zone.

The effects of BIODAC on the plant (canary grass and kidney bean) growth in the acid, neuter and basic soils were determined by detecting of the time of grain germination, height of plant and weight of produced biomass. No side effects were observed, on the contrary, in the acid soil the highest and weight of plants were major in the presence of BIODAC (probable due to it buffer property) as compared with control study without it (Fig. 1). It may be useful to remediate and increase productivity of acid soil. We did not detect the inhibitory effect of BIODAC to plant growth but these granules contain the kaolin with $Al^{3+}$ ions that (at certain concentration) may provoke the inhibition in the plant growth [8–10]. Evidently, this effect depends of the quantity of applying BIODAC and the using of this product in soil must be limited by reasonable quantities.

So, BIODAC using (at reasonable limits and under the controlled condition) did not affect the growth of plants and microorganisms, therefore, it may be applied as carrier to microbial inoculation of soils.

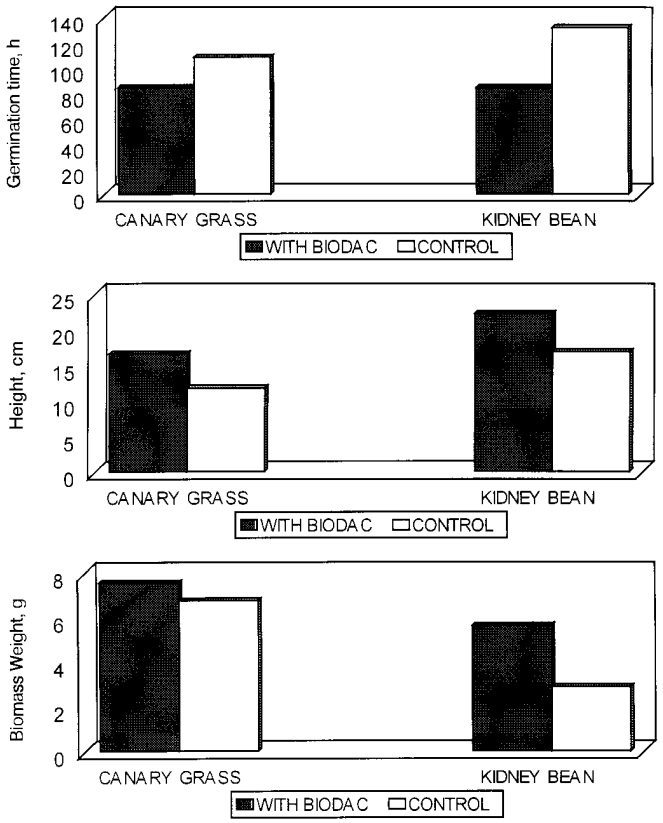


*Fig. 1.* Evaluation of effect of BIODAC 12/20 on the plant (canary grass and kidney bean) growth in the acid soil by detecting of: Top,—the time of grain germination; Middle,—height of plants and Bottom,—weight of produced biomass.

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