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Biodegradation of Diuron in Airlift Reactor by *Variovorax* sp. SRS16

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Abstract – *Diuron, is a substituted-urea herbicide considered as priority pollutant of water due to the persistency and high toxicity to aquatic organisms. In this work, the bacterium Variovorax sp. SRS16 was cultured in airlift reactor operated in batch mode with suspended cells, measuring diuron elimination en detoxification capability by standard toxicity test. Moreover, cell adhesion to inert supports was studied. Results indicated that cell growth and herbicide elimination are related, reaching higher biodegradation rates than previously reported in non-aerated reactor. In addition, biolite and sand particles supported biofilm formation, but the stress forces developed during reactor operation produced biofilm detachment.*

Keywords*: Airlift Reactor, Biodegradation, Diuron, Aerobic Bacterium, Biofilm*

I. Introduction

Diuron, N-(3,4-dichlorophenyl) N,N-dimethylurea, is a substituted-urea herbicide that has been in use in the 1950s on many agricultural crops and non-crops areas [1]. This compound is considered to be persistent in the environment (one month to one year) and due to their chemical characteristics it can be found in soil, sediments groundwater and superficial water [2]. Diuron has been included as a priority substance in the Water Framework Directive *(EU, 2001)*. Moreover, there are some studies that have shown the high toxicity to phytoplankton and aquatic invertebrates [3].

Otherwise, is well known the ability to degrade herbicides by specific communities of microorganisms. Therefore, a strategy for remediation of herbicide contamination in environment is the biomagnification using bacteria. A bacterium namely *Variovorax* sp. SRS16, which is capable of using the herbicide as a carbon, nitrogen and energy source, was isolated [4].

The main objective of this work was to measure the biodegradation of diuron by *Variovorax* sp. SRS16 under aerobic conditions at bioreactor scale. In the same way, it was study the capability of biofilm formation on inert support in order to explore possible application in three phase bioreactors. Different concentrations of carbon and nitrogen sources were used for the growing in airlift reactor. This is the first report in which this strain was cultured in a laboratory scale reactor.

II. Materials and Methods

Biodegradation was assayed in laboratory reactor using suspended and supported cells. Batch operation cycles of 48 hours were conducted with biomass without inert support in order to measuring diuron elimination rate, biomass growth and detoxification level reached. Once this series of assays was completed, it was explore biofilm formation by addition of biolite to reactor following its operation during 2 months. Previously it was studied biofilm growth using biolite and sand particles but in erlenmeyers and orbital shaker.

II.1. Microorganism and Culture Media

Variovorax SRS16 was isolated from agricultural soil and proved that can mineralize diuron and linuron [5], [6]. This strain was supplied by Dr. S. Sorensen (GEUS Institute-Denmark). Growing in Erlenmeyer for inoculum preparation was carried out using R2B complex medium without diuron at 20ºC and 150 rpm.

For biodegradation assays it was used a minimal medium containing 2g/L of succinate as carbon source, and 1-3 mg/L de diuron as nitrogen source. pH 7.1-7.2 was adjusted initially. Air was supplied at 1 L/min.

II.2. Airlift Reactor

The bubble column with internal circulation loop (airlift) was constructed in glass with a working volume of 1 L (height 0.45 m and 0.07 m of internal diameter). The raiser with 0.05 m of diameter was located at 0.06 m on the bottom of the column. Air was injected at the bottom of the column, across a perforated plate and establishing an up-flow of gas-liquid mixture in the raiser. On the top it was located a condenser working with cold water to prevent evaporation.

II.3. Analytical Procedures

II.3.1. Biomass Concentration

Biomass was measuring by optical density at 600 nm

(OD_{600}) and correlating with cell dry weight by a calibration curve, in which 1g/L of biomass correspond to 3.874 units of OD_{600}.

II.3.2. Succinate and Diuron Concentration

Liquid samples containing diuron were pretreated by solid extraction phase (SPE) for cleaning and concentration prior to HPLC-UV analysis, carried out using Varian ProStar system

Diuron was eluted at 22.5 min using a non-isocratic elution method consisting of a constant flow of 0.25 mL/min, initial water-acetonitrile proportion of 73:27 and final 100:0. The column used was a Kromasil C18 150 x 4.60 mm, 5 µm (Phenomenex). UV detection was done at 220 nm.

Succinate was measuring with a Supelcogel C610H 30 cm x7.8 mm (Supelco) at 210 nm. Elution was done at isocratic flow of 0.7 ml/min of deionized water with 0.1% v/v of H_3PO_4.

II.3.3. Toxicity Test

Liquid sample filtered by 0.45 µm membrane was incubated with cells of the green algae *Pseudokirchneriella subcapitata* under continues lighting at 23 ºC using microplates. Due to the high sensitivity of algae by the herbicides, samples were diluted 1:10 with algal culture medium prior to incubation. Procedure was done a according to OCDE Test Guide 201. Algae and culture medium was purchased from Microbiotest (Belgium). Algal growth was following by optical density at 620 nm by a microplate reader.

III. Results and Discussion

III.1. Biodegradation in Airlift

Evolution of biomass, succinate and diuron concentrations during the growing in airlift is shown in Fig. 1. Here it can be see that biomass growth reach to a stationary value of 1 g/L near to 30 hour of cultivation.

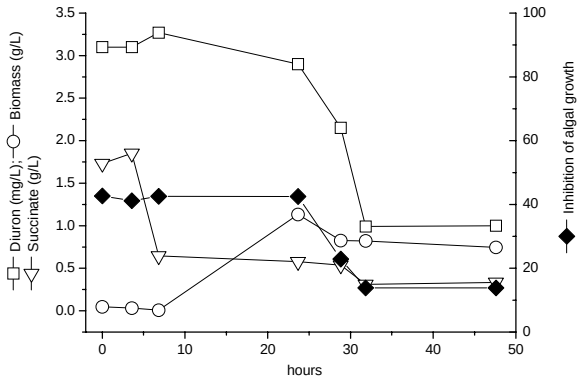

Fig. 1. Evolution of biomass, diuron, succinate and toxicity with cultivation time in a 2L airlift reactor

Carbon source was not completely depleted, due to succinate concentration remains constant and above 0.5 g/L at process times longer than 30 hours.

Diuron elimination is lower than biomass growing. Only after 20 hours occurred the most important drop of herbicide concentration.

Herbicide elimination rate in this lineal section of 25-30 hours was 0.234 mg diuron/L.h. While corrected by the biomasss concentration, this is 5.1 mg diuron/g-biomass.h. In relation to cellular growing, it was obtained a biomass yield of 0.041 g-biomass/mg succinate. The diuron elimination rate obtained in airlift is higher than the reported values of 0,012 mg diuron/L.h measuring in Erlenmeyer flask [5]. Nevertheless, *A.Globiformis* D47 reached a degradation rate of 0.291 mg diuron/L.h in Erlenmeyer at 20ºC. [5]

Toxicity test confirm the analytical values, because to the most important detoxification occurred after 24 hour of cultivation in the bioreactor. Initial toxicity of diluted samples was constant and around 40-45% of algal growth inhibition until 24 hours, while this level decreased to 10% at 30 hours.

III.2. Biofilm Formation

SEM microscopy (Fig. 2) indicated that bacteria could form biofilm on Biolite® and sands, but it was highly dependent on the stress forces developed in fluidization of supports. This image was taken after 1 month of growing in orbital shaker, using same culture media but instead to airlift it was cultured in Erlenmeyer flask under slow agitation and media renewal every 1 week.

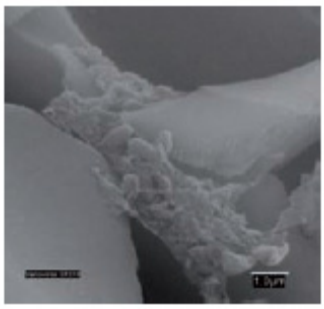

Fig. 2. Biofilm formation of *Variovorax* sp SRS16 on inert support

Growing and biofilm formation occurred only in this condition in both supports (biolite and sand granules). When support was added to airlift reactor, biofilm was not developed even after 2 months of reactor operation. Succinate and diuron consumption were measured but they was due to suspended cells instead to the attached biomass. Results obtained indicated that bioflim growing was unable in airlift, probably due to the shears forces.

IV. Conclusion

Variovorax SRS16 was able to degradate diuron in airlift bioreactor. Furthermore, it was observed that the

degradation rates achieved were quite high compared with the obtained at small-scale trials without air supply. In addition, bioflim formation did not thake place in airlift due to the high stress forces developed by friccion.

Acknowledgements

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