Effect of Drying on Bioremediation Bacteria Properties

F. Weekers, 1 Ph. Jacques, 2 D. Springael, 3 M. Mergeay, 3 L. Diels, 3 and Ph. Thonart, * 1

1 University of Liege, Walloon Center for Industrial Biology, Bat. B 40 - 4000 Sart-Tilman, Liege, Belgium; 2 Faculty of Agricultural Sciences of Gembloux, Bio-industries Unit, Passage des Déportés, 2-5030 Gembloux; and 3 Flemish Institute for Technological Research (VITO) Boerentag, 200 - B2400 Mol. Belgium

ABSTRACT

Bioremediation bacteria with drought-resistance characteristics were selected and compared to a collection of 10 strains selected only for their bioremediation properties. Twenty-six strains were selected from dried diesel-polluted soil, and they exhibit a better level of survival during drying, compared to collection bioremediation strains (two orders of magnitude difference). The lyophilization process does not affect the strains' ability to grow on xenobiotic compound when measured immediately after drying. However, collection bioremediation strains selected only for their bioremediation properties lose up to 80% of their properties when stored at 25°C for 15 d, but the strains selected for their drought resistance lose their properties to a lesser extent during the same period. The maximal growth rate and the rate of xenobiotic degradation of the still-active cells are not affected by the drying process.

Index Entries: Biodegradation; drought resistance; selection; maintenance of properties.

INTRODUCTION

The estimated number of contaminated industrial sites in the European Union is significant (150,000), as well as in the rest of the world (over 1,500,000 leaking underground storage tanks estimated in the United States alone) (1). Different techniques have been developed for the remediation of these sites (2). In situ bioremediation is one that has already been applied, but that deserves further development. The use of microbial products in

* Author to whom all correspondence and reprint requests should be addressed.
the bioremediation processes, however, is controversial and, in most cases, is being abandoned. These products usually have high efficiency in vitro, but competition, predation, lag phase, heavy metals copollution, and so on, make them less competitive than autochthonous strains when used in situ (3,4). However, in some cases of specific recalcitrant compound pollution, the use of appropriate starter cultures can readily boost the clean-up process (5). These starter cultures are mostly available in ready-to-use dry form, commercially distributed.

In these cases, it is important to have good knowledge of suitable techniques for the production and the conditioning of the starters. The drying process and its direct influence on the properties of the product constitute a bottleneck between the production chain and the in situ use of the bacteria. The final product must have a high survival ratio and maintain a high level of biodegradation activity.

To ensure a high level of survival after drying, the technique must be adapted to respect the cells’ integrity. The kinetics of water activity ($a_w$) variation is a very important factor (6–8) for the viability of bacteria subjected to a drying process. Since slower decrease of the $a_w$, down to a limit threshold, affords higher survival ratio, the drying methods should be designed to allow slow water depletion.

Survival after drying and stability over time of the surviving fraction are necessary, but not sufficient, conditions for a starter culture to be competitive (9). In addition, the degradation properties must be maintained in the surviving cells. Actually, ensuring genetic stability after drying and during preservation is a problem, since the viability of the cells after preservation may not correlate with the full maintenance of all properties. Plasmid-encoded degradation activities may be lost at high frequencies during drying of a culture, although little loss of viability occurs (10). Changes in various properties have been reported, especially during inadequate lyophilization (11–13). Although Lang and Malik (10) found a loss of properties in their strains, they could not detect any plasmid loss.

To quantify the biodegradation ability of a bacterium, the rate of substrate uptake ($-dS/dt$), i.e., degradation rate, is an important parameter to monitor (14). When comparing the influence of drying on biodegradation properties, one should also look at the maximal growth rate in different instances, because significant bacterial growth is of prime importance in a bioremediation process.

This laboratory specializes in large-scale drying of sensitive microorganisms of industrial interest. In this context, the fundamental phenomena accompanying the drying of the cells are studied. This paper reports the selection of bioremediation strains according to their resistance to the drying process, the characterization of their degradation properties, and the influence of the drying process on their survival, as well as on the maintenance of their degradation properties.