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Biodegradation of Cyanide by Rhodococcus Strains Isolated in Malaysia

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Abstract. Five strains of locally isolated *Rhodococcus* species from various sources in Malaysia were evaluated for their cyanide biodegradation potential. The partially characterized *Rhodococcus* UKMP-5M was used as reference strain and its ability to degrade cyanide in the form of KCN was tested at various concentration ranging from 3 mM to 15 mM. Whole cells with biomass amounting to 1 g L^{-1} (optical density equivalent to 0.500) of the strain were able to rapidly degrade almost 50 % of 12 mM cyanide over a period of 10 hours. The degradation was carried out at 30 °C, pH 7 and with an agitation at 160 rpm in the absence of added organic and inorganic substances. In addition, by employing the identical amount of biomass and other experimental conditions, the different strains of locally isolated *Rhodococcus* species were compared for their ability to degrade cyanide. It was revealed that *Rhodococcus* UKMP-5M had the highest percentage of degradation followed by *Rhodococcus zopfii*, *Rhodococcus* sp1, *Rhodococcus* NAM81 and *Rhodococcus* sp2 which corresponded to 47.78 %, 29.17 %, 23,61 %, 18.33% and 11.67 % degradation respectively.

Keywords: *Rhodococcus*, cyanide, biodegradation, biotransformation

1. Introduction

Cyanide is a carbon nitrogen radical, which may be found in a wide variety of life forms and their large scale presence in the environment is attributed to the manufactured sources which are used extensively in industries. Cyanide is commonly found as a contaminant in wastewaters through release from metal finishing, electroplating and coal coking which are the crucial factors that contribute to the bulk occurrence of cyanide in the environment (Dash *et al*., 2009). Cyanide in various forms is highly toxic, carcinogenic, and mutagenic.

Hence, control and remediation of cyanide–contaminated water is desired because of the potential hazards associated with cyanide. There are several conventional methods used in treating effluents containing cyanide before discharging it into the environment. The most common ones are the alkaline chlorination, sulfur oxide/air process and hydrogen peroxide process. Although these methods of treatment can be used in detoxifying free cyanide bearing waste, they pose significant drawbacks as listed below:

- The disability to treat cyanide that is complexed with metals.
- High costs of reagents, equipments, maintenance and royalty payments.
- The generation of unfavorable by-product such as chlorinated compounds.

Thus, with rapid growth in many applications that utilize cyanide, it is necessary to use bioremediation as the most promising platform to prevent contamination of soils and wastewater. Many attempts to develop biological processes for the detoxification of cyanide have been concentrated on cyanide-degrading fungi (Meyers *et al*., 1991) and several studies have been established on the use of bacterial strains such as *Klebsiella, Pseudomanas, Acinetobacter, Bacillus, Burkholderia* and *Alcaligenes* (C.M. Kao et al., 2003).

Meanwhile, it is also interesting to note that the utilization of *Rhodococcus* in degrading cyanide is rather promising. *Rhodococcus rhodochrous* in particular showed remarkable potential in degrading cyanide even

at a concentration of 260 mg L^{-1} and can be considered as source for the isolation of cyanidase (Keusgen *et al*., 2001). Rhizosphere microbial community, *Rhodococcus* sp which was isolated from two cyanogenic plants apparently utilizes oxidative reactions for cyanide degradation as well (Y.L. Hong, 2007). It is also a fascinating revelation to note that two facultative autotrophs, both actinomycetes, of the genus *Nocardia* and in another case a gram-positive filamentous organism, probably again an actinomycete, have also been found to be capable of growing on cyanide as a carbon and nitrogen source. This indicates that *Rhodococcus*, an actinomycete, has a huge potential to degrade cyanide as it belongs to the genus *Nocardia* (Ezzi *et al*., 2004). Hence, in the present work, the feasibility of using locally isolated *Rhodococcus* strains for their potential in cyanide biodegradation is assessed.

2. Materials and Methods

2.1. Microorganisms and Growth Conditions

Strains of locally isolated *Rhodococcus* species were kindly provided by the Culture Collection Unit, Institute of Bio-IT Selangor. To the production medium containing (g L^{-1}) nutrient broth, 8.0; glucose, 8.0; and L-proline, 10.0, 1 ml (2 % v/v) of 24 hours preculture of *Rhodococcus* UKMP-5M (optical density equivalent to 1.000-1.200) grown in 8.0 g L^{-1} nutrient broth were inoculated. It was incubated at 30 °C with an agitation at 160 rpm. The cells were harvested at early stationary phase (48 hours) by centrifuging the culture at 4400 rpm for 30 minutes at 4 °C, washed twice with 100 mM potassium phosphate buffer (pH 7.0) and resuspended in the same buffer to be stored at 8 °C until further analysis.

2.2. Biotransformation of Cyanide

An amount of 2.5 ml of the suspended cells (1.0 g L^{-1} dry cell weight, optical density equivalent to 0.5) were transferred into screwcap bottles and cyanide in the form of KCN were added to a final concentration of 3, 6, 9, 12 and 15 mM respectively and left to shake at 160 rpm and 30 °C. Hundred microlitre of samples were removed, diluted to 10 mL and assayed for remaining cyanide at 2, 4, 7, 10, 24 and 30 hours respectively by employing a modified barbituric acid method (Nagashima, 1977).

Identical culture conditions were employed to cultivate the other locally isolated Rhodococcus strains. For the purpose of strain comparison study, the percentage of cyanide degradation was determined after 10 hours of incubation with an initial cyanide concentration of 12 mM following the similar experimental method as mentioned above.

All chemicals were of analytical grade obtained from various commercial sources. Control experiments were established without bacterial cells and experiments were carried out in triplicate and experimental errors were estimated and depicted with error bars. A one way ANOVA test (95 % confidence interval) was used to evaluate differences between the tested *Rhodococcus* strains. Then, the strains were ranked and the best strain with the highest percentage of degradation was obtained based on Duncan analysis.

3. Results and Discussion

The strain was unable to grow aerobically on minimal medium with KCN as the sole source of carbon and nitrogen which was in agreement with previous reports (Adjei and Ohta, 1999; Meyers *et al*., 1991 and C.M. Kao *et al*., 2000). The addition of initial inoculums perhaps were unable to sustain a basal metabolism and needed ample of energy and time in order to produce enzymes that could metabolize the highly toxic substrate, KCN (Dhillon and Shivaraman, 1999).

On the contrary, the biomass was able to rapidly degrade up to 47.78 % of 12 mM cyanide after 10 hours of incubation as depicted in Figure 1. It was clearly noticeable that 63.33 % of 3 mM KCN was degraded within 10 hours of incubation followed by 50.56 % of 6 mM KCN, 48.52 % of 9 mM KCN, 47.78 % of 12 mM KCN and 1.11 % of 15 mM KCN. The bacterium was not able to metabolize or transform 15 mM KCN as the percentage of degradation decelerated drastically which was attributed to the intolerable toxicity level of cyanide. As illustrated in Figure 1, it was rather apparent that the percentage of degradation became constant after 10 hours of incubation. This may be due to the limited capability of the resting cells amounting to 1 g L^{-1} to express sufficient amount of cyanide degrading enzymes to support continuous degradation and eventually to completely break down cyanide. Meanwhile, cyanide concentrations in the control system

remained almost unchanged throughout the experimental process indicating that the depletion of cyanide is primarily due to biodegradation activity.

The cyanide degrading activity was present even in the cells grown in the nutrient broth without KCN. Cells cultivated to their stationary phase were better equipped by accumulating factors that enable them to handle variety of environmental stress than exponential phase (Adjei and Ohta, 1999). For that reason, the strains of *Rhodococcus* were harvested at their early stationary phase (48 hours) and later used as resting cells for cyanide degradation.

Although the strain was not capable of growing exclusively by utilizing KCN as the sole source of carbon and nitrogen, it was able to directly transform KCN by means of resting cells (Meyers *et al*., 1991). This may be due to the presence of a low level of constitutive synthesis of the cyanide degrading enzymes (Adjei and Ohta, 1999). Moreover, *Rhodococcus* UKMP-5M was also observed to grow in medium containing nitriles and the whole cells were able to hydrolyze nitriles to its corresponding carboxylic acid and ammonia (Sjahrir *et al*., 2010). It was fascinating to notice that the predicted protein sequence from the gene encoding the enzyme cyanide hydratase that has been isolated and sequenced from *Fusarium lateritium*, *Fusarium solani*, *Gloeocercospora sorghi* and *Leptosphaeria maculans* demonstrated strong homology to all available nitrilase and cyanide dihydratase sequences. On top of that, it was also a refreshing revelation to discern the fact that the enzyme cyanide dihydratase that has been characterized from *Psedomonas stutzeri* AK61 was more closely related to nitrilase compared to that of cyanide hydratase. Furthermore, the active site for cyanide hydratase enzyme and nitrilase activity in the protein was the same for *Fusarium lateritium* (Nolan *et al*., 2003). Hence, the enzyme present in the culture of *Rhodococcus* UKMP-5M that transformed nitriles to carboxylic acid and ammonia may be capable of converting KCN as well.

Within the *Rhodococcus* strains, *Rhodococcus* UKMP-5M exhibited the highest percentage of cyanide degradation which was 47.78 % whereas *Rhodococcus* sp2 displayed the least percentage of degradation, merely 11.67 %as portrayed in Figure 2. Appreciable percentages of degradation were observed for *Rhodococcus zopfii*, *Rhodococcus* sp1 and *Rhodococcus* NAM81 which corresponded to 29.17 %, 23.61 % and 18.33 % respectively. Having stated that, *Rhodococcus* UKMP-5M was selected for further experiments since the strain evidently possesses huge potential to be useful in cyanide bioremediation.

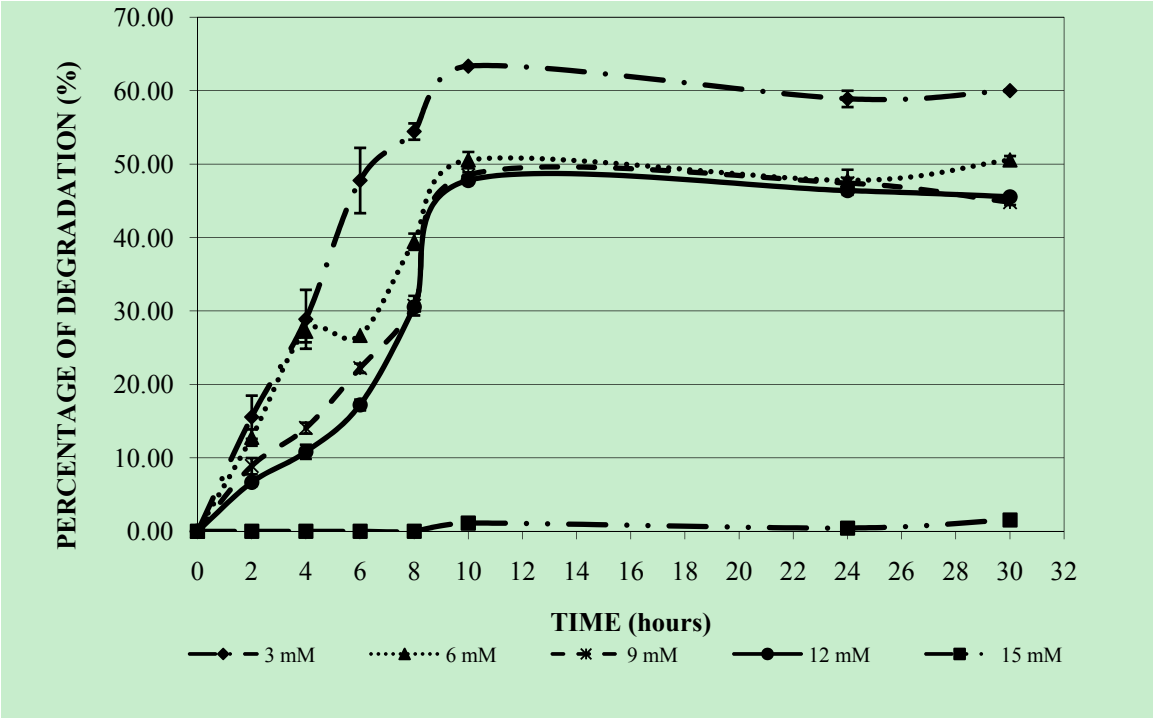

Figure 1: Cyanide degradation by Rhodococcus UKMP-5M

*All the values are the means of three independent experiments and the standard error bars are as shown.

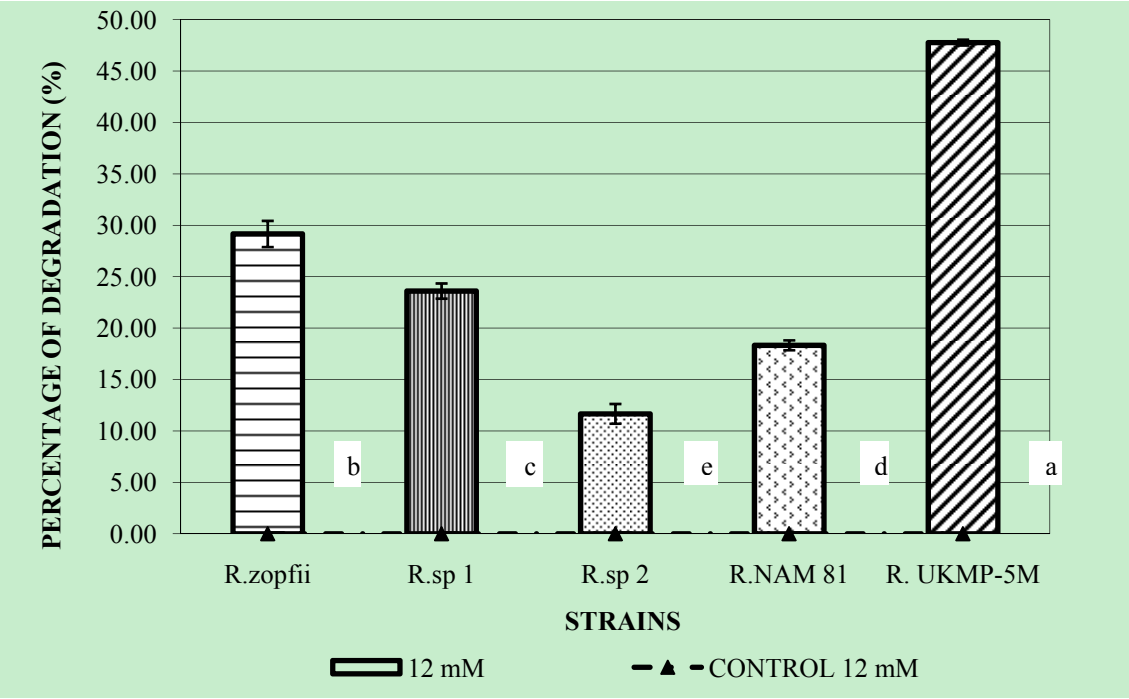

Figure 2: Cyanide degradation by different strains of locally isolated Rhodococcus species

*All the values are the means of three independent experiments and the standard error bars are as shown. Statistically significant differences ($P < 0.05$) were observed among the tested strains of *Rhodococcus* species.

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