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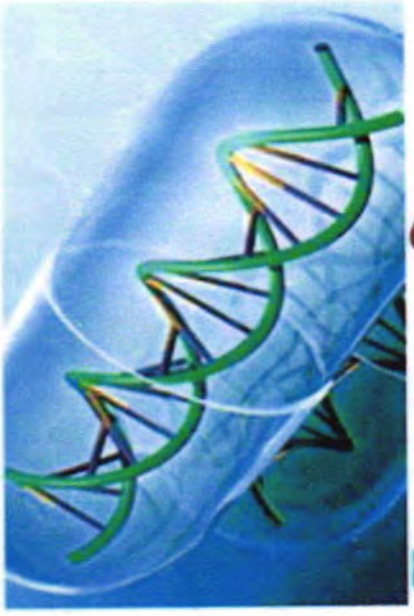
Proceeding
Enzyme Technology for Eco-Friendly Industry



Serpong, Tangerang
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Introduction

Enzymes are biocatalysts which accelerate a certain (bio) chemical reaction and found in every cell of all living beings, from simple single cellular organisms to highly complex multicellular organisms. Enzymes play an important role in our live, not only in our own metabolism, but also in other processes sustaining our daily activities. They are used to assist the production of the food we eat and contribute to the care of our health by providing therapeutic agents or sensitive and specific diagnostic tests. Thus it has high commercial and industrial value.

Enzymes also play an important role in 'green industry'. Instead of using more hazardous chemicals, enzymatic process is a more environmentally friendly choice. For example, instead of using chlorine for the bleaching processes, protease is a 'greener' choice for detergent as well as pulp and paper industries.

In addition, products more environmentally friendly processed are now in higher demands, due to tighter environmental regulations, especially in the developed countries. Cases involving rejections of Indonesian exported products by the environmentalists because the manufacturing processes were considered not environmentally friendly should never have occurred, had the decision makers applied stricter laws and had the industrialists had more awareness on environmental issues. Enzymes, therefore, would be one of the answers.

The data from our market research consultant indicated that in 2004 the value of enzyme demand in Asia Pacific was 640 millions US dollar, where 387 millions US dollars is associated with industrial enzymes and 253 US dollars with specialty enzymes used in the pharmaceutical, research and diagnostic fields. Although the pattern is less predictable, Indonesian domestic demands seem to increase quite steadily between 2001-2005, reaching more than 3500 tons in total in 2005. The highest demand seems to be from the detergent industry, consisting on average 15% of total. The rests were used in other industrial sectors, including leather processing, cattle feed, textiles, pulp and paper, as well as food and pharmaceuticals. Unfortunately, the whole amount is still completely imported.

Considering the ever increasing application of enzymes in industries, worldwide as well as domestically, it is only reasonable that researchers in the field of biocatalysts as well as decision makers start to think about this problem. In Indonesia, where industries are not yet closely linked with the research and academic world, research products would always be merely research products unless the decision makers assert their authority in policy making, supporting the application of domestic research products in national industries. Therefore, in order to bring the industrialists, enzyme researchers and decision maker closer together, we organised the Conference on Industrial Enzyme Biotechnology 2010 (CIEB 2010), which was held in Tangerang on 3 – 6 August 2010, with title **Enzyme Technology for Eco-Friendly Industry**.

Tangerang, 3 August 2010

Director of
Center for Bioindustrial Technology

Witono Basuki

Content

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The Uptake of Cadmium Ions and Its Influence on The Growth and Production of Some Secondary Metabolites in Shoot Cultures of *Solanum melongena*

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ABSTRACT

The aim of this study was to examine the response given by shoot cultures of *Solanum melongena* upon uptaking of cadmium ions. From the results of this research, it could be stated that shoot cultures of *Solanum melongena* were able to grow in the media containing 40-160 μM cadmium ions, with the growth index greater than three; while application of higher concentration of that ions will result in the death of the cultures. The cultures were able to remove 20.2-34.4% of the ions from media containing 40-160 μM of the ions and accumulated them in their biomass. Application of 120 μM cadmium ions to the media caused a decrease in total free phytosterols content (50.2%) of the cultures.

Keywords: Cadmium ion, shoot culture, *Solanum melongena*, phytosterols.

INTRODUCTION

Cadmium is a chemical element with the symbol Cd and atomic number 48, occurs with zinc ores. Cadmium is used largely in batteries and pigments, for example in plastic products, and known to cause cancer. Cadmium is also a potential environmental hazard. Human exposures to environmental cadmium are primarily the result of the burning of fossil fuels and municipal wastes. However, there have been notable instances of toxicity as the result of long-term exposure to cadmium in contaminated food and water. In the decades following World War II, Japanese mining operations contaminated the Jinzu River with cadmium and traces of other toxic metals. As a consequence, cadmium accumulated in the rice crops growing along the riverbanks downstream of the mines. The local agricultural communities consuming the contaminated rice developed Itai-itai disease and renal abnormalities, including proteinuria and glucosuria. Cadmium is one of six substances banned by the European Union's Restriction on Hazardous Substances directive, which bans carcinogens in computers (Anonim, 2008).

Cadmium and zinc are in the same group on the periodic table, contain the same common oxidation state (+2), and when ionized are almost the same size. Due to these similarities, cadmium can replace zinc in many biological systems, in particular, systems that contain softer ligands such as sulfur. Cadmium can bind up to ten times more strongly than zinc in certain biological systems, and is notoriously difficult to remove. In addition, cadmium can replace magnesium and calcium in certain biological systems, although these replacements are rare (Anonim, 2008).

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Plant cell cultures, as well as its intact plant can give certain responses on the presence of certain metal ions in the medium. Cultures of *Chlorella vulgaris* could remove 75% of 8 μM copper ions from the medium (Herman, 1995). Hairy root cultures of *Armoracia rusticana* could remove 62.4% of the copper ions from initial value of 330 μM copper ions (Zikmundova, 1995). Copper tolerant callus cultures of *Nicotiana tabacum* regenerated plants that were able to grow in the presence of 100 μM copper ions (Gori et al., 1998).

The production of sesquiterpenoid phytoalexins in cell suspension cultures of *Datura stramonium* have been induced by metal ions, including cadmium ions (Threfall and Whitehead, 1989).

The results of preceding researchs demonstrated that introducing metal ions, including cadmium ions, into plant cell cultures could induce, increase, or inhibit the production of some secondary metabolites in that cultures.

EXPERIMENTAL

Materials

The shoot cultures of *Solanum melongena* were cultivated on modified Murashige and Skoog media with the addition of 2 $mg.L^{-1}$ benzyladenine. Cultures were maintained in the light using Philips TL40W at 25^0C. Subculturing of the stock-shoots was performed every 4 weeks (Indrayanto et al., 1996).

In order to study the accumulation of cadmium ions and its influence on the production of some secondary metabolites, shoot cultures were transferred to a series of 20 mL media containing different concentration of cadmium ions and cultivated for 4 weeks under the same conditions prior to harvesting. For each cadmium ions concentration, 15 bottles of ±1-1.5 gram fresh weight of shoots each were used. After incubation the cultures were harvested, weighed, bulb dried at 40^0C, and powdered.

Growth study

The growth of shoot cultures was expressed in *growth index* (*GI*), defined as the ratio of final fresh weight and initial fresh weight of each culture. The GI was measured for each bottle.

Analysis of cadmium ions

Cadmium ions in the biomass were determined by using ICP-AES after appropriate digestion of 0.1 mg powder using 2.0 ml concentrated HNO_3 followed by dilution with distilled water up to 10.0 ml or diluted further as required (Chen, 1993). Quantification was performed by measuring the emission intensity of cadmium at its maximum wavelength. The concentration of cadmium ions was calculated from the linear regression equation of standard.

RESULTS AND DISCUSSION

Growth study

The results of observation of the growth rate expressed in growth index (GI) and the decrease of sucrose concentration in media before and after harvesting were shown on Table 1, Figure 1 and 2.

Table 1. The growth index of the shoot cultures of *Solanum melongena* aged 4 weeks and the decrease of sucrose concentration in media before and after harvesting

Concentration of Cd^{2+} (μM)	Replication	Growth index	Decrease of sucrose concentration (%)
40	15	3.80±0.84	64.50±0.74
80	15	6.32±1.58	30.20±0.61
120	15	6.80±2.00	28.42±0.58
160	15	4.58±1.84	24.51±0.55

The growth index was expressed as average ± deviation standard, change in sucrose concentration as % average ± deviation standard.

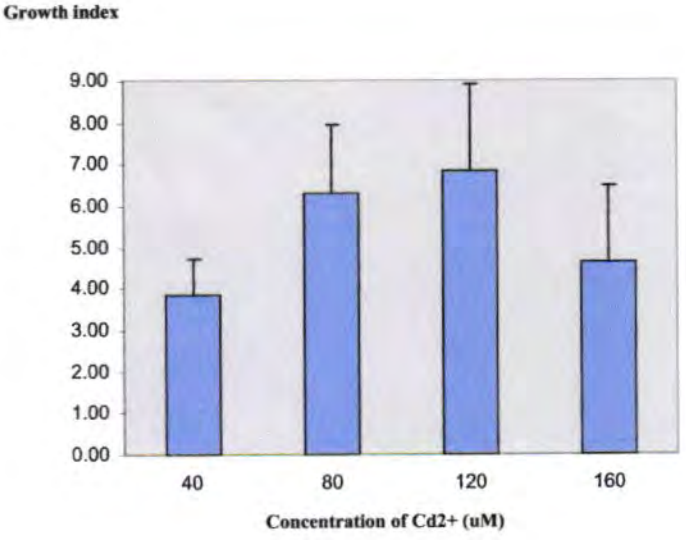

Figure 1. Graph of growth index variation of *Solanum melongena* shoot cultures aged 4 weeks with the increasing concentration of Cd^{2+}

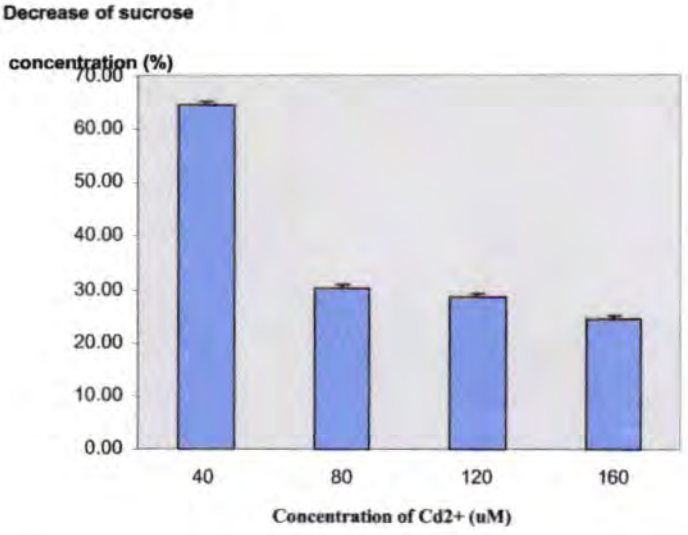

Figure 2. Graph of the decrease of sucrose concentration in media with variation of Cd^{2+} concentration

Table 1 and Figure 1 showed that the shoot cultures of *Solanum melongena* were able to grow in the media containing cadmium ions concentration up to 160 µM with the growth index greater than three. The growth rate increased with the increasing concentration of cadmium ions, reached its maximum at the concentration of approximately 120 µM and decreased afterwards. It was seen from Table 1, Figures 1 and 2, that the growth rate was not always inversely proportional to the decrease of sucrose concentration in media. It was possibly due to the difference in absorption of nutrient other than sucrose by the cultures.

Analysis of phytosterol(s)

The results of the analysis of steroidal glycosides(s) using anisaldehyde-H_2SO_4 as a spraying reagent were shown on table 2.

Table 2. Analysis of steroidal glycoside(s) using tin layer chromatography (TLC)

Concentration of Cd^{2+} (µM)	Steroidal glycoside(s)	
	Result	Rf
40	+	0.63 (green), 0.71 (yellow), 0.83 (violet)
80	+	0.63 (green), 0.71 (yellow), 0.83 (violet)
120	+	0.63 (green), 0.71 (yellow), 0.83 (violet)
160	+	0.63 (green), 0.71 (yellow), 0.83 (violet)

+ indicates that a substance being identified is existing, Rf is retardation factor

Table 2 showed that producing steroidal glycoside(s) in their cells, the shoot cultures of *Solanum melongena* gave certain spot colour after being sprayed with anisaldehyde-H_2SO_4.

Accumulation of cadmium ions and determination of the total free sterols content in biomass

The results of the observation of accumulation of cadmium ions and total free sterols content in biomass of *Solanum melongena* cultures aged 4 weeks were demonstrated on Table 3, Figure 3 and 4.

Table 3. The accumulation of cadmium ions and the total free sterols content in biomass of *Solanum melongena* cultures aged 4 weeks

Concentration of Cd^{2+} (µM)	Replication	Concentration of Cd^{2+} in biomass (µg/g)	Concentration of total free sterols (µg/g)
40	15	3.6±0,2	568±98
80	15	52.0±1.4	450±75
120	15	107.2±4.6	421±60
160	15	135.9±2.7	324±87

Concentration of cadmium ions and total free sterols was expressed in µg/g dry weight of biomass

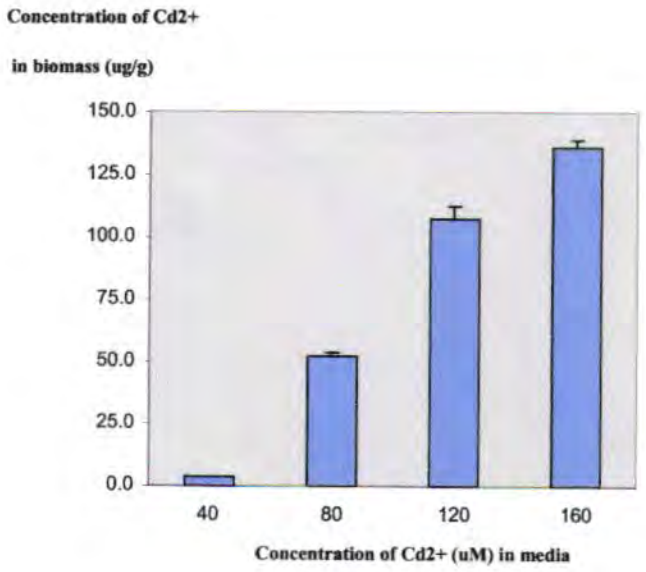

Figure 3. Graph of concentration of Cd^{2+} in biomass with variation of Cd^{2+} concentration in media

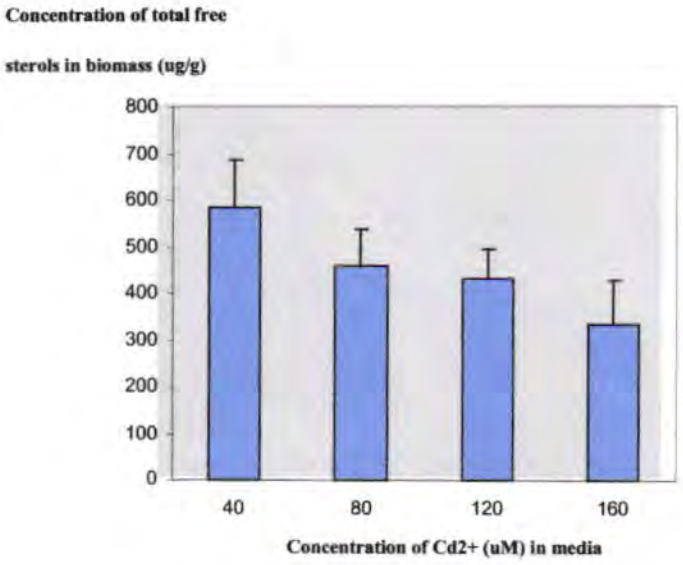

Figure 4. Graph of concentration of total free sterols with variation of Cd^{2+} concentration in media

Table 3 and Figure 3 showed that the shoot cultures of *Solanum melongena* were able to grow to the media containing cadmium ions concentration up to 160 μM and able to remove 20.2-34.4% cadmium ions from media containing 40-160 μM of the ions and accumulated them in their biomass. Application of 120 μM cadmium ions to the media caused a decrease in total free phytosterols content (50.2%) of the cultures. Moreover, Table 3 and Figure 4 also demonstrated that application of cadmium ions concentration caused a decrease in total free sterols content of the shoot cultures of *Solanum melongena*. It was the higher the cadmium ions concentration, the higher the decrease in total free sterols content.

CONCLUSIONS

It could be concluded from the research, that:

1. The shoot cultures of *Solanum melongena* were able to grow in the media containing cadmium ions concentration up to 160 μM and able to remove 20.2-34.4% of cadmium ions from media containing 40-160 μM of the ions and accumulated them in their biomass. Application of 120 μM cadmium ions to the media caused a decrease in total free phytosterols content (50.2%) of the cultures.
2. Introducing cadmium ions into shoot cultures of *Solanum melongena* could inhibit the production of total free phytosterols in that plant cell cultures.

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