電子工程學系
Department of Electronic Engineering
香港中文大學 The Chinese University of Hong Kong

Toxicity study of Sanson Pico Biocatalyst

SHATIN NT HONG KONG · 香港 新界 沙田 · TEL 電話 +852 3943 8486, 3943 8270 · FAX 圖文傳真 +852 2603 5558 · EMAIL 電郵 dept@ee.cuhk.edu.hk · WEBSITE 網址 http://www.ee.cuhk.edu.hk

Project Summary

In this work, we investigate the toxicological profile of a microbiological preparation, Sanson Pico Biocatalyst, later referred to as Pico Powder or Pico. Sanson Pico Biocatalyst consists of two yeast strains (*Dekkera anomaia, Pichia farinosa*) and three bacterial species (*Bacillus amyloliquefaciens, Pediococcus acidilactici, Pediococcus pentosaceus*), of which all are certified as biosafety level 1 organism. Acute and chronic toxicological profile on invertebrate, as well as the ecotoxicity profile on economic crops and invertebrate, was characterized. Due to the powerful protease and lipase activities of Sanson Pico Biocatalyst, there is also concern whether it would cause harm to the unfertilized or fertilized eggs of fish. Since many of the intended usages of Sanson Pico Biocatalyst involve significant discharge of this preparation through sewage, these questions must be clearly addressed before the commercialization of the Sanson Pico Biocatalyst.

Two seawater invertebrates *artemia salina* (brine shrimp), *neanthes arenaceodentata* (polychaete worm) and a freshwater invertebrate, *Paratya compressa improvisa* (freshwater shrimp) were chosen for toxicity test. All the species are of millimeter scale and show no appreciable risk to human being. Seawater invertebrates were supported by artificial seawater made with inorganic salts. Diluted suspension of Sanson Pico Biocatalyst was added to the water at various concentrations. The number of motile and immotile individuals was counted every day. At the end of experiment period (>14 days), the test organisms were collected by filtering and/or sieving and had their dry weight measured. For toxicity in an agricultural context, 28-day repeated dose toxicity study on soybean was carried out. Seeds of soybean were allowed to bud. When the soybean plantations reached a steady state of growth, Sanson Pico Biocatalyst suspensions, at various concentrations, were applied to the soil as part of the routine watering. Growth profile as indicated by viability, height and stem diameter were recorded for analysis.

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Materials and methods

Artemia salina mortality test

Dehydrated cysts of *Artemia salina* were used. Stage I larvae nauplii were separated from non-attached cysts based on phototaxis and then transferred by a Pasteur pipette into beakers containing the filtered natural seawater. Toxicity test was performed by adding approximately 15 larvae to each well of a 24-well polystyrene plate containing 1 mL of filtered natural seawater with suspensions of different concentrations of Sanson Pico Biocatalyst (0.01, 0.1, 1.0, 10, 100 mg/mL). The plates were stored at 20C for 48 h with a 16:8-h light/dark cycle. All of the tests were performed in triplicate. After 48 h, the larvae were collected from the wells, rinsed and transferred into new plates with clean filtered natural seawater. The numbers of dead larvae were counted under a stereoscopic microscope. Non-motile larvae were considered dead. Percent mortalities are corrected for natural mortality in controls using the Abbots formula,

$$\rho = (PI - C)/(1 - C)$$

where PI is the observed mortality rate and C is the natural mortality. Lethal concentration (LC_{50}) is calculated using the probit analysis.

Swimming speed alternation test

After 48 h of exposure of the *Artemia salina* larvae to different concentrations of Sanson Pico Biocatalyst, the swimming path and behavior were recorded using a stereoscopic microscope equipped with an infrared video camera. The apparatus was placed inside a light-shielded box to exclude ambient light. *A. salina* larvae were dark-adapted for 2 min before the video recording. The swimming behavior was recorded.

Effect of Sanson Pico Biocatalyst on Paratya compressa improvisa

Toxicity effect of Sanson Pico Biocatalyst on *Paratya compressa improvisa* was carried out exactly the same way as *Artemia salina* except seawater was replaced by freshwater.

Effect of Sanson Pico Biocatalyst on Neanthes arenaceodentata

Test chambers were aerated and illuminated continuously during the 20-day test period. Worms were fed every 2 d, and water was renewed every 3 d. After 20 d, samples were sieved through 0.5-mm filters, and the number of surviving worms recorded. Survivors were placed in pre-weighed foil in an oven until they reached a constant weight.

Effect of Sanson Pico Biocatalyst on soybean

Soybean was grown in soil medium containing graded concentrations of Sanson Pico Biocatalyst (0.01, 0.1, 1.0, 10, 100 mg/kg). Plant growth parameters were recorded. Four uniform weight soybean seeds surface sterilized by 0.1% $HgCl_2$ solution for 2 min followed by thorough rinsing with distilled water. Soybean shoot and root lengths and root biomass were recorded on 14, 28, 42, 56, 70 and 84 days after sowing. The shoot and root were dried in an oven at 65°C for 48 hours.

Statistical analysis

Data collected were analyzed using MatLab to determine significance between mean values. Duncan's multiple range test was used to compare differences among means. Significant level was chosen at $p > 0.05$.

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Results and discussion

Artemia salina (brine shrimp)

The toxicity of Sanson Pico Biocatalyst was tested against *Artemia salina*. No lethality cases were observed. The lowest dose causing lethality (LDLO) and the lowest dose causing a toxic effect (TDLO) are both beyond 100 mg/mL. Based on the toxicity data (Fig. 1), Sanson Pico Biocatalyst is considered to be non-toxic to *Artemia salina*. The effect of Sanson Pico Biocatalyst on the locomotive behavior of *Artemia salina* was also tested. A recording system was implemented to tracking the swimming behavior and speed alternation over a set period of time. As shown in Fig. 1, the locomotive behavior of *Artemia salina* was not impaired by Sanson Pico Biocatalyst. In the control experiments, the shrimp moved with a speed of 1.02 ± 0.22 m/min, whereas the test subjects generally moved with a faster speed. In particular, at 100 mg/mL Sanson Pico Biocatalyst, the shrimp moved with a 7.5% increase in speed at 1.10 ± 0.32 m/min (p<0.001).

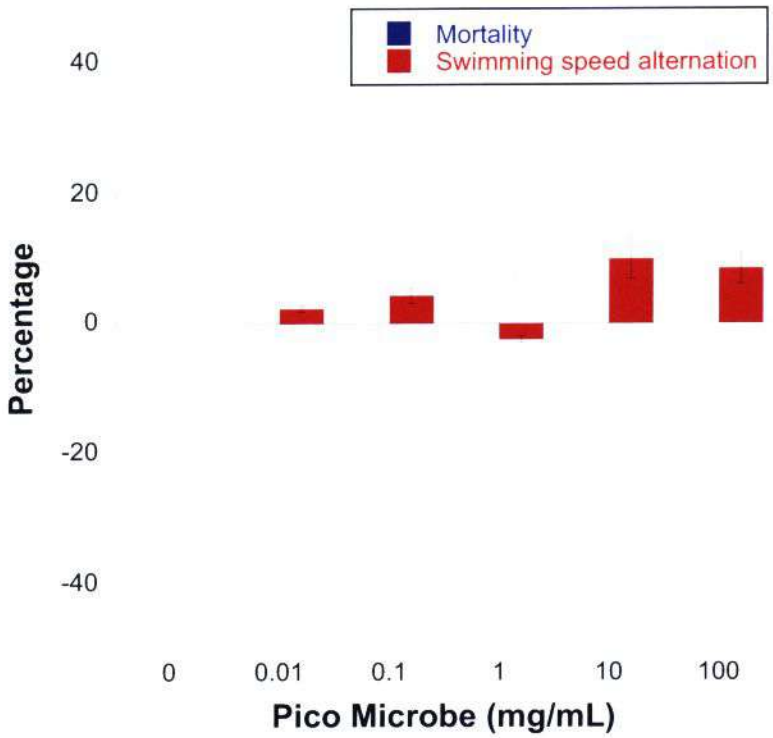

Figure 1. Toxicity effect of Sanson Pico Biocatalyst on *Artemia salina* shrimps.

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The long-term toxicity effect of Sanson Pico Biocatalyst was tested on *Artemia salina* by measuring the length of shrimp larvae (Fig. 2). The *Artemia salina* embryos exposed to 0.01 mg/mL Pico Microbe Biocatalyst do not result in significant difference in larval length compared with the control averaging at a larval length of 750 μm ($p < 0.05$). As the Pico Microbe Biocatalyst concentrations increased to around 1 mg/mL, a 20% increase in larval length was recorded.

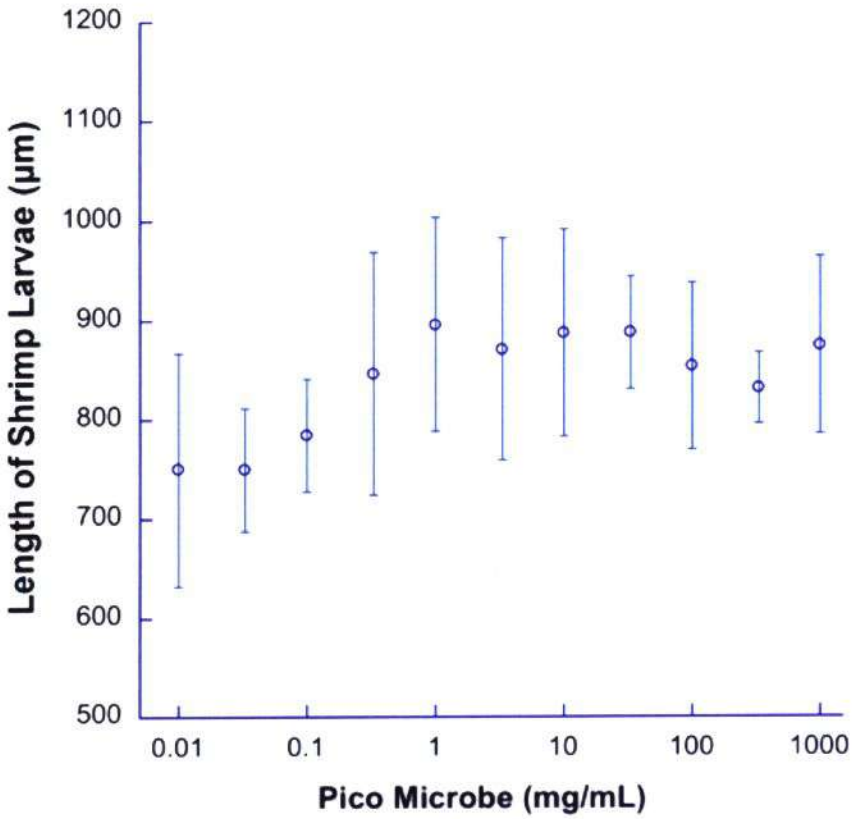

Figure 2. Toxicity effect of Sanson Pico Biocatalyst on the length of *Artemia salina* shrimp larvae.

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***Paratya compressa improvisa* (freshwater shrimp)**

The toxicity of Sanson Pico Biocatalyst was tested against *Paratya compressa improvisa*, a type of freshwater shrimp. No lethality cases were observed. The lowest dose causing lethality (LDLO) and the lowest dose causing a toxic effect (TDLO) are both beyond 100 mg/mL. Based on the toxicity data (Fig. 3), Sanson Pico Biocatalyst is considered to be non-toxic to *Paratya compressa improvisa*. The effect of Sanson Pico Biocatalyst on the locomotive behavior of *Paratya compressa improvisa* was also tested. A recording system was implemented to tracking the swimming behavior and speed alternation over a set period of time. As shown in Fig. 3, the locomotive behavior of *Paratya compressa improvisa* was not impaired by Sanson Pico Biocatalyst. In the control experiments, the freshwater shrimp moved with a speed of 1.31 ± 0.35 m/min, whereas the test subjects generally moved with a faster speed. In particular, at 100 mg/mL Sanson Pico Biocatalyst, the shrimp moved with a 11.1% increase in speed at 1.45 ± 0.41 m/min (p<0.001).

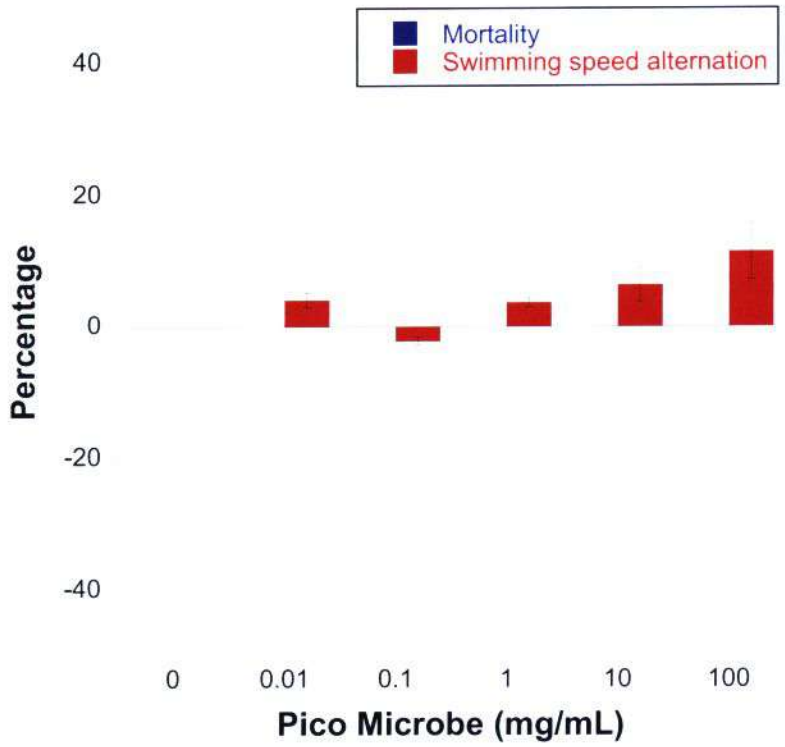

Figure 3. Toxicity effect of Sanson Pico Biocatalyst on *Paratya compressa improvisa* shrimps.

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The long-term toxicity effect of Sanson Pico Biocatalyst was tested on *Paratya compressa improvisa* by measuring the length of shrimp larvae (Fig. 4). The *Paratya compressa improvisa* embryos exposed to 0.01 mg/mL Pico Microbe Biocatalyst do not result in significant difference in larval length compared with the control averaging at a larval length of 1410 μm ($p < 0.05$). As the Pico Microbe Biocatalyst concentrations increased to around 1 mg/mL, a 25% increase in larval length was recorded.

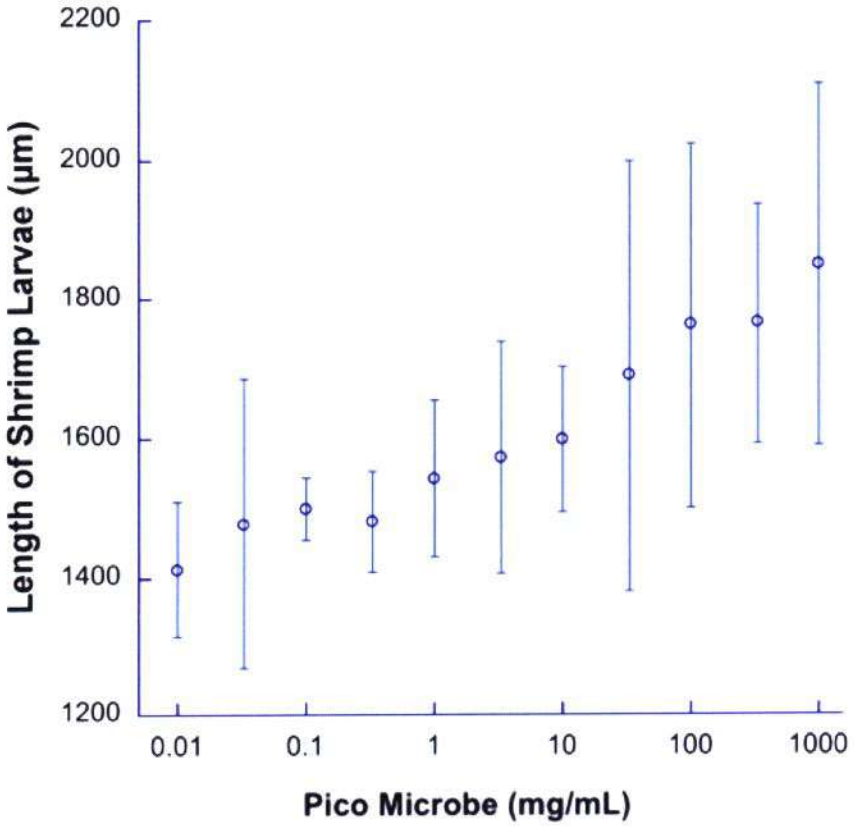

Figure 4. Toxicity effect of Sanson Pico Biocatalyst on the length of *Paratya compressa improvisa* shrimp larvae.

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***Neanthes arenaceodentata* (polychaete worm)**

The long-term toxicity effect of Sanson Pico Biocatalyst was tested on *Neanthes arenaceodentata* polychaete worms by measuring the dry mass (Fig. 5). The polychaete worms larval worms exposed to 0.01 mg/mL Pico Microbe Biocatalyst do not result in significant difference in dry mass compared with the control averaging at a dry mass of 242 ±42 mg ($p < 0.05$). When the worms were subjected to higher concentrations of Sanson Pico Biocatalyst, varying effects on the dry mass were observed. Both stimulatory and inhibitory effects were noted.

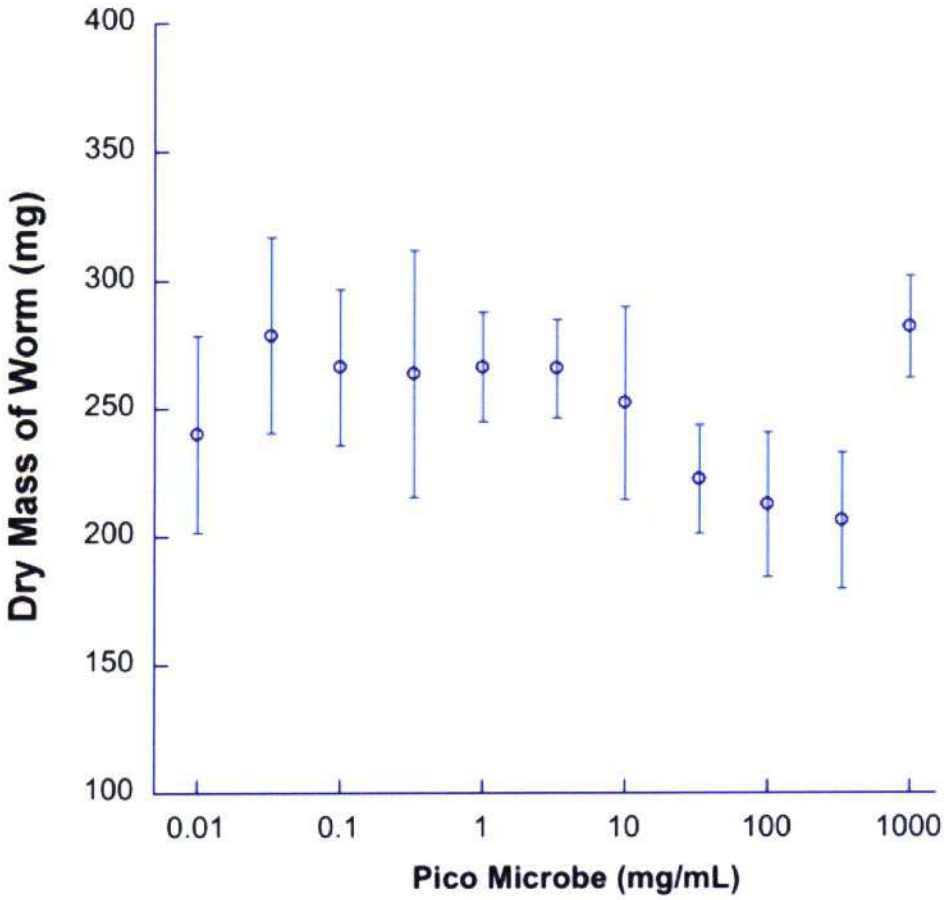

Figure 5. Toxicity effect of Sanson Pico Biocatalyst on the dry mass of polychaete worm.

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Soybean

The shoot height of soybean has experienced a stimulatory effect upon exposure to Sanson Pico Biocatalyst (Fig. 6). At 1 mg/kg and 10 mg/kg, no significant difference was observed among the soybean shoot heights. The height remained in the range of 25 to 26 cm. At a dosage of 100 mg/kg and 1000 mg/kg of Sanson Pico Biocatalyst, a significant boost in height was observed with an average of 30.5 cm. At an even higher concentration (10,000 mg/kg), the shoot height reached 36.8±3.9 cm.

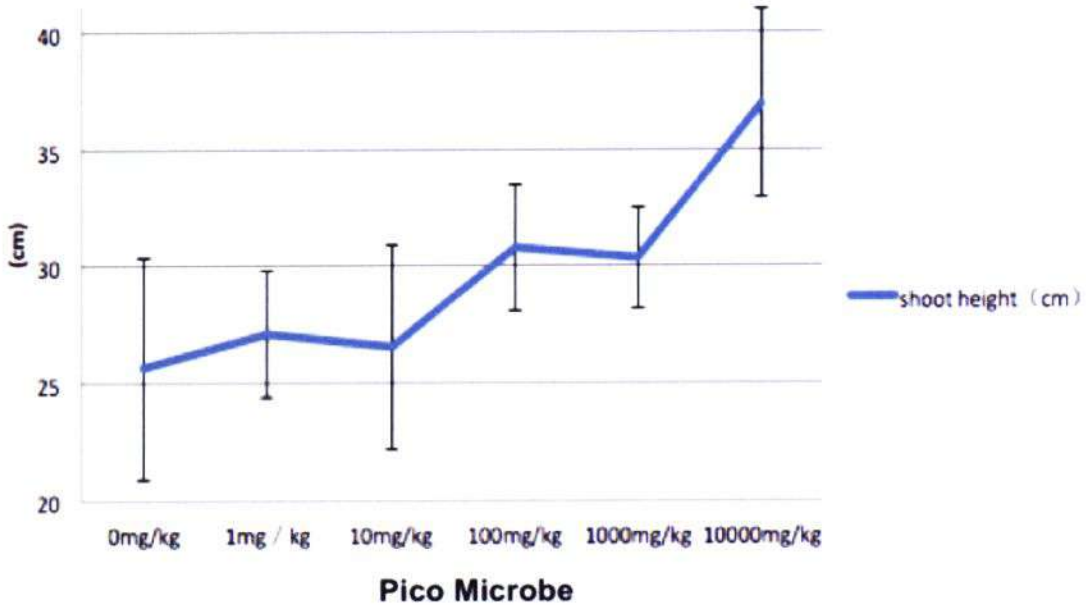

Figure 6. Toxicity effect of Sanson Pico Biocatalyst on the shoot length of soybean.

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Conclusion

Sanson Pico Biocatalyst has been applied to a number of invertebrates, vertebrates and plants to test its toxicity profile. No mortalities have found been to any of the tested organisms exposed to a range of Sanson Pico Biocatalyst concentrations up to 10,000 mg/kg in soil or 100 mg/mL in water. It is of interest that Sanson Pico Biocatalyst has demonstrated a positive growth effect on the tested organisms. For instance, soybeans grew faster in the presence of Sanson Pico Biocatalyst. Brine and freshwater shrimp both swim more erratically and faster when Sanson Pico Biocatalyst were added. Based on the toxicity studies, Sanson Pico Biocatalyst exhibited no toxic effects in the tested organisms and may mitigate the negative effect of secondary pollution as observed in the conventional cleaning and maintenance of grease interceptors.

Prof. Douglas Yung
Department of Electronic Engineering
Division of Biomedical Engineering
The Chinese University of Hong Kong
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SHATIN NT HONG KONG · 香港 新界 沙田 · TEL 電話 +852 3943 8486 3943 8270 · FAX 圖文傳真 +852 2603 5558 · EMAIL 電郵 dept@ee.cuhk.edu.hk · WEBSITE 網址 http://www.ee.cuhk.edu.hk