



# Sustainable Biochemical Engineering Lab

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Cell-Free Metabolic Engineering for Circular Bioeconomy



## Research Interests

“Responsible Consumption and Production” is among one of 17 main goals of the United Nation Sustainable Development Goals (SDGs) in 2030. Based on the concept of the circular bioeconomy, our lab develops facile and cost-effective **cell-free enzymatic cascades** to valorize organic solid wastes into value-added products. For example, we use microalgae to treat phosphorus-containing sewage wastewater, and the inorganic polyphosphate produced by microalgae can be used to regenerate ATP and can serve as raw materials for the bio-industry. In addition, we also convert solid organic wastes into non-toxic, value-added products by microbial proteases. In this project, protein wastes can be converted into microbial growth media for fermentative protein production. The circular bioeconomy models established in our lab can effectively **reduce the volume of organic solid waste** and **produce value-added, environmentally benign goods**.

## Selected Projects

### 1. Biorefinery of microalgal polyphosphates for the circular bioeconomy

The diagram illustrates the biorefinery of microalgal polyphosphates. It starts with **N-deficient conditions** leading to the production of polyphosphate (polyP<sub>n</sub>). The process involves enzymatic conversion by **PPK1** and **PPK2** to produce **polyP<sub>(n-1)</sub>**. This polyphosphate is then used to regenerate **ATP** from **ADP** via **PPK2**. The ATP is used for the synthesis of **Adenosine** from **AMP** by the enzyme **ADK**. Micrographs show that phosphorus is not accumulated in some cells but is accumulated in others. Fluorescence images show algae-derived polyP stained with DAPI (blue) and long-chain polyP visualized using DAPI.

### 2. Heterogeneous expression of protease to convert organic solid waste

The diagram shows the production of protease from organic waste. **Fish waste** is hydrolyzed to **Fish waste hydrolysate** using **Protein hydrolysis**. **Bagasse** is hydrolyzed to **Acidic hydrolysate** using **Acidic hydrolysis**. The hydrolysates are used for **Genetic modification** and **Induction of heterologous protein expression** in **Bacillus megaterium**. The process involves **Cell autolysis** at **60°C, pH 9** to produce **Purified enzymes** (Aminopeptidase). The induction of heterologous protein expression is shown on **LB agar plate** and in **GFP-expression** assays, comparing **Induction (-)** and **Induction (+)**.

## Selected Publications

Gicana, R. G., Yeh, F. I., Hsiao, T. H., Chiang, Y. R., Yan, J. S., & Wang, P. H. (2022). Valorization of fish waste and sugarcane bagasse for Alcalase production by *Bacillus megaterium* via a circular bioeconomy model. *Journal of the Taiwan Institute of Chemical Engineers*, 135, 104358.