



# Illumination optimization strategies to enhance hydrogen productivity and light conversion efficiency for photo-fermentation by *Rhodobacter sphaeroides* KKU-PS1 using a concentrated multi-substrate feedstock

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## ABSTRACT

With the use of biotechnology, hydrogen can be produced from wastewater rich in short-chain fatty acids. A previous study revealed the ability of *Rhodobacter sphaeroides* KKU-PS1 to produce biohydrogen from substrates mimicking succinate fermentation effluent. However, the process still requires optimization. Before illumination optimization, due to high concentration of the effluent, various effluent dilution factors ranging from 10 to 100 were compared, and the optimal dilution factor was determined to be 50. Light-emitting diode (LED) setups consisting of bands and tubes were compared, and various illuminated surface-to-volume ratios (S/V) were obtained. LED tubes were subsequently used for light intensity optimization in the range of 5–23 klux, revealing optimum light intensity at 15 klux, yielding 2202 mL H<sub>2</sub>/L and 13.8 mL H<sub>2</sub>/L/h as the cumulative hydrogen and maximum output rate, respectively. The lighting protocol at 15 klux and with a 6h–6h light-dark cycle improved the total light conversion efficiency by up to 3.1%. The study successfully optimized the process, with results rivalling those of a previous study using malate.

## 1. Introduction

Energy recovery from waste, which is one of the cornerstones of the circular economy, could replace traditional fossil-based energy resources [1]. Energy from waste can be recovered in the form of hydrogen which, thanks to its clean combustion product and high energy density, is seen as a potential energy carrier by means of biotechnological methods [2–4]. Hydrogen and hydrogen-based fuels are among key pillars of decarbonization in achieving Net Zero Emission by 2050 especially for heavy transportation and industry [5]. However, not all hydrogen production routes contribute to decarbonization, particularly

those that involve fossil fuels or coal-fired generation due to high overall greenhouse gases (GHG) emission [6]. Hydrogen generated via electrolysis that utilizes renewable energy such as wind and solar power, often dubbed as green hydrogen, is currently the preferred route of production [7]. Albeit given lesser attention, biological hydrogen production, also known as biohydrogen, could prove pivotal to achieve Net Zero Emission, especially with benefit of circular economy if generated from industrial wastewater [6,8].

Biohydrogen can be produced via acidogenic anaerobic digestion or also known as dark fermentation, biophotolysis, microbial electrolysis cell (MEC), and photofermentation, each having different substrates of preference [9–11]. Therefore, compatibility of different industrial

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