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***Enterobacter ludwigii* strain IF2SW-B4 isolated for bio-hydrogen production from  
rice bran and de-oiled rice bran**

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**Abstract** This research is aimed to study fermentative bio-hydrogen producing bacteria isolated from rice bran (RB) and de-oiled rice bran (DORB) wastes of oil extracting industry where RB used as renewable biomass. Therefore, the present research was carried out to isolate different bacterial species from RB and DORB wastes and their along with microscopic and biochemical characterization. Secondary screening was performed to analyze their efficiency of bio-hydrogen production (BHP) under optimum temperature of  $37\pm 2^{\circ}\text{C}$  and at initial pH 6. Total 115 bacteria were isolated seasonally from RB and DORB wastes. Highest production of  $545\pm 5$  ml/l  $\text{H}_2$  bio-hydrogen was obtained in second season by RB II-6 was confirmed as *Enterobacter ludwigii* strain IF2SW-B4 based on 16S rRNA gene sequencing. Its specific hydrogen production rate (SHPR) is 3.29 ml/g substrate/h in 3<sup>rd</sup> day and 1.89 ml/g substrate/h after 6<sup>th</sup> day of BHP whereas DORB II-4 gave  $295\pm 5$  ml/l  $\text{H}_2$  production and SHPR of 1.82 ml/g substrate/h in 3<sup>rd</sup> day and 1.02 ml/g substrate/h in 6<sup>th</sup> day of production.

**Keywords:** BHP; rice bran; de-oiled rice bran and SHPR.

## 1. Introduction

There is a lot interest in developing is technologies to produce bio-hydrogen among the various renewable energy sources. It is a clean alternative energy carrier fuel having low-cost for processing (Das and Veziroglu, 2001). It is carbon-free energy source with high energy yield ( $120\text{MJ kg}^{-1}$ ) which is almost three times higher than most hydrocarbon fuels (Kapdan and Kargi, 2006; Rittmann, 2008). This is an important aspect considering the increased consumption of fuel in 21<sup>st</sup> century. Heavy use of hydrocarbon fuel has led to change in global climate. Currently, only 2% of hydrogen is being utilized and will be increasing by 8-10% by 2025 (Winter, 2005; Azman et al., 2015). Among the various hydrogen production processes, biological methods are most efficient and eco-friendly process. The biological process is known to be less energy intensive than chemical or electrochemical (Hallenbeck and Benemann, 2002; Nath and Das, 2007). Biological processes mostly include photo-fermentation, dark fermentation or hybrid processes (Chang, 2002). Dark fermentation is a carbon neutral process for production of bio-hydrogen from breakdown of carbohydrate rich substrates. Facultative and obligate anaerobic bacteria includes different species of *Clostridium*, *E.coli*, *syntrophobacteria*, and enteric bacteria such as *Enterobacter* (Tanisho, 2001; Hallenbeck and Ghosh, 2009; Rajhi, 2013). Under aerobic conditions, these bacteria degrade organic substrates by oxidation to produce to produce molecular hydrogen ( $\text{H}_2$ ) (Das and Veziroglu, 2001; Nath and Das, 2004). Fermentative bacteria have high evolution rate of hydrogen. It is a complex process and affected by many factors such as amount of substrate, nitrogen, metal ion, temperature, and pH (Levin et al., 2006; Wang et al., 2007). Lignocelluloses biomass is an abundant and a good resource for energy production. Agriculture based industries generate waste containing cellulose, hemicelluloses and lignin. This waste can be used as a good source for bio-hydrogen production. Biomass is used as substrate because of their frequent availability and cost effective (Kapdan and Kargi, 2006). It is a difficult for microorganisms to extract valuable carbohydrates from these sources. Thus, pre-treatment of substrates is necessary for increasing hydrogen production (Kumar et al., 1995; Chen et al., 2001;

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