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Enzymatic bio-refinery for the sequential production of bio-diesel, bio-ethanol, bio-hydrogen and bio-methane using tannery fleshing, sludge and molasses waste

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The concept of bio-refinery has been demonstrated first time in the present study by utilizing the residue after each bio-fuel recovered as a feed stock for further fuel recovery sequentially to produce biodiesel, bioethanol, bio-hydrogen and bio-methane. The fat was recovered (80%) from tannery fleshing waste and subjected to lipase based enzymatic transesterification and recovered biodiesel as fatty acid methyl esters (FAME) (60-80 L/kg of tannery fleshing) under optimized pH, catalyst, mixing and reaction conditions. The FFA profile and FAME was confirmed using FTIR. The residue of biodiesel was primarily glycerol, which was further anaerobically co-digested with molasses and recovered crude bioethanol (30–50 L/L of mixed glycerol and molasses) employed with specific ethanol seed sludge pre-fermented with molasses at an optimized pH of 4.5. Separate acidophilic hydrogenic and methanogenic seed inocula was pre-acclimatized and activated from the preheated STP sludge with appropriate anaerobic nutrients and maintained at optimum pH conditions of 5.5 and 6.5 respectively. Acidogenic/hydrogenic seed sludge and ethanol residue waste in the mixing ratio of 1:1 was used for biohydrogen production. The maxima hydrogen production was observed in the pH range of 5.5 to 5.7 as 0.3Nm L/gm of VS removed that was coincided with rod-shaped bacteria through SEM analysis. The residue after hydrogen production was used to generate methane using separate pre-acclimatized methanogenic seed sludge and the methane maxima was found to be 0.3 Nm.L/gm of VS_r. This was consistent with cocci-shaped microbial SEM structures at the pH range of 6.3 to 6.5 which was confirmed using two stage anaerobic CSTR for ameliorated hydrogen and methane generation at varying organic loading rates (OLR) and hydraulic retention time (HRT). Therefore, this study successfully demonstrated the concept of bio-refinery to extract multiple bio-fuels sequentially from the high fat, carbohydrate and protein rich organic tannery solid wastes to achieve zero solid discharge (ZLD), to reduce greenhouse gas emissions, with high carbon foot prints.

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Water hyacinth: A promising step towards sustainable future in bio-energy and *Lupinus termis* salinity stress alleviation

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In order to reduce conventional energy sources dependence, biofuels derived from renewable sources have received extensive interest. Water hyacinth is a promising source in the production of clean renewable energy. In this study, water hyacinth as a promising potential energy crop and its role in alleviating salinity stress of *Lupinus termis* were studied. The physicochemical parameters of water hyacinth and the conventional feedstock (cow dung) were analyzed for nutrients and minerals content. Maximum cumulative biogas and methane production were recorded for water hyacinth compost. Salinity stress led to a highly significant decrease in all growth criteria of *Lupinus termis*. The application of water hyacinth compost to the soil resulted in a highly significant stimulation in the *Lupinus termis* growth parameters. Photosynthetic pigments showed that salinity level led to a highly significant decrease in chl.a and chl.b of *Lupinus termis*. The effect of water hyacinth compost on the photosynthetic pigment content was reflected in a highly significant increase in chl.a and chl.b with a highly significant decrease in carotenoids content at seedling stage. There was an increase in the activities of catalase and peroxidase at this seedling stage under salinity stress. Moreover, a highly significant increase in the ascorbate content was detected by high salinity. Finally, transmission electron micrograph (TEM) changes in the ultrastructure of leaves of 30- days old plantlets of *Lupinus termis* were studied. This study suggested that water hyacinth not only promising biofuel source but also enhances the growth parameters and alleviate salinity stress of *Lupinus termis*.

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