# Abstract

Currently, one of the major issues at the global level is to meet rising fuel demand along with reductions in non-renewable source based energy generation and $CO_2$ emissions. Hydrogen is widely acknowledged as a "carbon-neutral energy carrier" with high potential to replace fossil fuels. Nevertheless, development of a holistic approach for hydrogen generation is quintessential towards achieving feedstock, environmental and economic sustainability. The present study embarks upon the realization of an integrated biorefinery perspective for biohydrogen production from microalgal biomass via dark fermentation (DF). Microalgae, *Scenedesmus obliquus* UTEX 393 was found to be a propitious feedstock for DF process. The biomass and carbohydrate accumulation of the microalgae were maximized via physico-chemical parameters optimization using single and multi-parameter optimization strategies. Multi-response optimization study resulted in maximum biomass and carbohydrate productivity of 491 mg $L^{-1}$ $d^{-1}$and 270 mg $L^{-1}$ $d^{-1}$, respectively at the initial pH 6.69, temperature 27.65 $^{o}$C, glucose concentration 3.33 g $L^{-1}$ and urea concentration 126.77 mg $L^{-1}$. The biomass and carbohydrate productivity were further enhanced to 560 mg $L^{-1}$ $d^{-1}$ and 309 mg $L^{-1}$ $d^{-1}$, respectively using airlift photobioreactor. The carbohydrate content under optimized process parameters was improved by 73.5% as compared to un-optimized growth conditions. Moreover, effect of $CO_2$ concentration study indicated that $CO_2$-air sparging of 5% v/v supported maximum microalgal growth and carbohydrate production with $CO_2$ fixation ability of 727.7 mg $L^{-1}$ $d^{-1}$. Application of a pretreatment step is often recommended to efficiently release the microalgal carbohydrates for enhanced bio$H_2$ yield. So further, the study aimed to identify suitable pretreatment condition by considering two types of microalgal biomass viz. *S. obliquus* UTEX 393 biomass (cultivated under optimized process conditions) and RIL microalgal biomass (dried microalgal biomass powder procured from Reliance Industries Limited, RIL, Mumbai, Maharashtra, India). In a biorefinery framework, lipids present in microalgal biomass were extracted and deoiled microalgal biomasses (DMBs) were processed with various pretreatment techniques. Thermo-acidic pretreatment (0.5N $H_2SO_4$, 121$^{o}$C for 30 min) documented highest carbohydrate recoveries of 87.5% and 85.6% with biohydrogen yields of 89.2 mL $g^{-1}$ VS and 42.5 mL $g^{-1}$ VS from *S. obliquus* UTEX 393 biomass and RIL microalgal biomass, respectively. The study was followed with integration of DF process with anaerobic digestion (AD) and microalgal cultivation (MC) to enhance the substrate energy recovery. By utilizing DF effluent of deoiled *S. obliquus* UTEX 393 biomass, methane production of 1060 mL $L^{-1}$ was achieved. Furthermore, *C. reinhardtii* UTEX 90 biomass production of 1.68 g $L^{-1}$ in DF effluent, affirmed the suitability of hydrogenic spent for MC. Total energy recovered (TER) from deoiled *S. obliquus* UTEX 393 biomass by DF, integrated DF-AD and integrated DF-MC processes were 7.7%, 23.2% and 20.5%, respectively. Beside carbohydrate, DMB also contain considerable amount of protein and microelements, thus could act as plausible co-substrate for organic wastes lacking nitrogen and various micronutrients essential for $H_2$ production. Addition of DMB (non-pretreated) to starchy wastewater (SWW) remarkably increased the bio$H_2$ production with maximum cumulative $H_2$ production of 1970 mL $L^{-1}$ attained at 3% w/v DMB.

Subsequent channelling of DF effluent (obtained from fermentation of DMB supplemented SWW) to AD and MC process resulted in maximum methane production of 1765 mL $L^{-1}$ and microalgal biomass production of 1.77 g $L^{-1}$, respectively. Consequently, overall substrate energy recovery augmented from 4.1% (DF process) to 15.7% and 10.1% in DF-AD and DF-MC coupled system, respectively.

**Keywords:** dark fermentation; microalgae; parameter optimization; carbohydrate productivity; deoiled microalgal biomass; pretreatment; carbohydrate recovery; biorefinery; biohydrogen; biomethane; energy recovery.