

Abstract

Energy demand is escalating continuously due to rapid industrialization and modernization of the world which leads to depletion of fossil fuels and increases the cost of fuels. Higher consumption of fossil fuels increases the emission of greenhouse gases (GHGs), which adversely impacts the biodiversity and environment. Hence, concern towards energy security and environment has shifted our attention towards developing alternative fuel from renewable resources. Biobutanol, bioethanol and biodiesel represent major liquid transportation biofuels among which biobutanol has greater energy density and is compatible with the existing transportation infrastructure unlike ethanol and biodiesel.

Biological butanol production from first generation feedstock makes the process economically unviable because of the high cost of the raw materials that incurs 60-70 % of the total production cost. In this milieu, the second generation (2G) feedstocks are an alternative to reduce the cost of the raw materials. Lignocellulosic feedstock is a potential source of sugars owing to the presence of a large percentage of cellulose and hemicellulose. Agricultural residues, energy crops, and agro-industrial wastes are attractive 2G feedstock for biobutanol production. Lignocellulosic biomass is sufficiently abundant and generates low net greenhouse emissions, thus can be an ideal precursor to produce biofuels.

In the present study, *Bambusa bambos* was used a raw material for biobutanol production which is rich in holocellulose content (~62 % w/w on the dry weight basis) and has no commercial value. India has the second largest diversity of bamboo in the world and produces 5.4 million metric tons of bamboo residues annually. Laccases from *Pleurotus djamor* and cellulases-xylanases from *Trichoderma reesei* RUT C30 were used for delignification and enzymatic hydrolysis of holocellulose, respectively. Enzymatic hydrolysis of laccase pretreated bamboo was optimized through central composite design (CCD) based response surface methodology (RSM) and maximum reducing sugar 529.53 mg/g reducing sugar was

obtained in 8 hours of saccharification time with 76.24 %, w/w saccharification efficiency. Simultaneous pretreatment and saccharification (SPS) of bamboo was carried out using enzyme cocktail of laccases from *Pleurotus djamor* and cellulases-xylanases from *Trichoderma reseei* RUT C30 for pretreatment and producing reducing sugar in single step to save energy and time. After optimization of SPS process using CCD based RSM maximum reducing sugar (503.11 mg/g) was obtained in 8 hours of incubation time with saccharification efficiency of 72.44 %, w/w. Reducing sugar obtained through separate pretreatment and enzymatic hydrolysis (SEH), and SPS were evaluated for butanol production using *Clostridium beijerinckii* ATCC 55025-E604. Fermentation studies showed that similar efficiency of fermentation of both SEH and SPS hydrolysate. Further optimization of butanol production from SPS hydrolysate was carried out and after optimization of the fermentation process maximum butanol 11.54 g/L was obtained with the yield of 0.231 g/g-reducing sugar with productivity of 0.192 g/L. h.

Keywords: Bamboo, Laccase, Pretreatment, *Clostridium beijerinckii*, Fermentation, Biobutanol