

Effects of inorganic salts on biomass production, cell wall components, and bioethanol production in *Nicotiana tabacum*

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Abstract The development of bioenergy through biomass has gained importance due to the increasing rates of fossil fuel depletion. Biomass is important to increase the productivity of bioethanol, and production of biomass with high biomass productivity, low lignin content, and high cellulose content is also important in this regard. Inorganic salts are important in the cultivation of biomass crops for the production of biomass with desirable characteristics. In this study, the roles of various inorganic salts in biomass and bioethanol production were investigated using an in vitro tobacco culture system. The inorganic salts evaluated in this study showed dramatic effects on tobacco plant growth. For example, H₂PO₄ substantially improved plant growth and the root/shoot (R/S) ratio. The chemical compositions of tobacco plants grown in media after removal of various inorganic salts also showed significant differences; for example, lignin content was high after Mg²⁺ removal treatment and low after K⁺ treatment and H₂PO₄ removal treatment. On the other hand, NO₃⁻ and H₂PO₄ treatments yielded the highest cellulose content, while enzymatic hydrolysis yielded the highest

glucose concentration ratio 24 h after NH₄⁺ removal treatment. The ethanol productivity after H₂PO₄ removal treatment was 3.95% (w/v) 24 h after fermentation and 3.75% (w/v) after 36 h. These results can be used as the basis for producing high-quality biomass for future bioethanol production.

Keywords Enzymatic hydrolysis, Fermentation, Inorganic salts, in vitro culture, *Nicotiana tabacum* L.

Introduction

This is because lignocellulosic biomass is non-edible, can be continuously reproduced, and is environmentally friendly because it does not emit greenhouse gases during production and use (Chandel et al. 2018). It is estimated that about 130 gallons of ethanol can be produced from 1.3 ton of biomass (Carroll et al. 2009).

Lignocellulosic biomass is mainly composed of cellulose, hemicellulose, and lignin, making it difficult for enzyme hydrolysis. That is why some pretreatment steps are essential to alter the physical and/or chemical structure of lignocellulosic biomass and easily convert polysaccharides into fermentable sugars, making cellulose more accessible (Kumar and Sharma 2017).

To effectively use lignocellulosic biomass, structural factors representing cellulose specific surface area, cellulose crystallinity, degree of polymerization, pore size and volume, and chemical factors related to the composition and content of lignin, hemicellulose, and acetyl groups must be considered (Zoghlami and Paës 2019).

Biomass is very important as a raw material for bioenergy, but the chemical composition of biomass is also important. Lignin imparts water resistance, firmness, and mechanical strength to the secondary cell wall, but interferes with the saccharification of cellulose. Lignin imparts water resistance, firmness and mechanical strength to the secondary cell

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