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Estimation of inhibitory effects of hemicellulosic wood hydrolysate inhibitors on PHA production by *Burkholderia cepacia* ATCC 17759 using response surface methodology

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HIGHLIGHTS

- Using response surface methodology to estimate the inhibitory effects of multiple inhibitors.
- Utilizing microbial growth and PHA content as response surfaces.
- Estimating synergistic inhibitory effects among select inhibitors.
- Monitoring the degradation and metabolism of inhibitors.

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ABSTRACT

Sugar maple hemicellulosic hydrolysate was utilized as a renewable feedstock for polyhydroxyalkanoates production by *Burkholderia cepacia* ATCC 17759. To estimate inhibitory effects of the hydrolysate, response surface methodology was utilized to analyze cell growth and PHA accumulation in the presence of multiple inhibitors. Mixture design was employed to study the correlation between the proportion of phenolics and total inhibition. The resultant models (R^2 as 92.42% and 93.14% for cell growth and PHA production, respectively) indicated syringic acid was the most inhibitory among three phenolics and synergistic inhibition was observed for the combinations of vanillin/syringic acid and vanillic acid/syringic acid. When furfural, levulinic acid, and acetate were also present during the fermentation, central composite design was employed. The regression model using 48 h cell growth as the response surface (R^2 = 87.82%) showed acetate was the most inhibitory. Additionally, strong synergistic effects were observed for the combinations of acetate/phenolics and levulinic acid/furfural.

1. Introduction

Polyhydroxyalkanoates (PHAs) are a class of polyesters synthesized and stored by various bacteria as intracellular carbon sources under metabolic stress or during unbalanced growth conditions (Hazer and Steinbuchel, 2007). Besides being completely biodegradable, PHAs exhibit comparable thermal and material properties to petrochemical-derived plastics and could be considered as potential alternatives to these recalcitrant materials (Madison and Huisman, 1999).

In order to lower the cost of PHA production, inexpensive and renewable feedstocks have been utilized as carbon sources (Choi and Lee, 1999; Du et al., 2012; Waller et al., 2012). As a potentially inexpensive wood-based feedstock, hemicellulosic wood hydrolysate is produced through a dilute acid hydrolysis of wood biomass and contains variable amounts of fermentable sugars composed primarily of xylose, rhamnose, mannose and glucose, as well as inhibitory compounds such as acetic acid, levulinic acid, furan derivatives and phenolic compounds (Amidon et al., 2008; Hu et al., 2010).

Burkholderia cepacia ATCC 17759 is a Gram-negative bacterium capable of producing short-chain-length PHAs including polyhydroxybutyrate (PHB), as well as copolymers of PHB and polyhydroxyvalerate (PHV) when provided with monomer precursors such as levulinic acid, which also inhibits cell growth at high concentrations (Keenan et al., 2004). Meanwhile, *Burkhoderia* strains are widely employed as bioremediation agents due to their ability to degrade toxic compounds such as polyaromatic hydrocarbons, polychlorinated biphenyls, furans and lignin monomers (Goris

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