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EFFECT OF THE ENVIRONMENTAL FACTORS ON BIOLOGICAL PRETREATMENT USING MICROBIAL CONSORTIUM

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INTRODUCTION

Biological resources such as agricultural and forestry residues have raised more interest as a potential feedstock for the production of low-cost ethanol production. However, the transformation of these biological sources into valuable-added products required pretreatment before fermenting microorganisms can convert them into ethanol.

Among various pretreatment methods, the biological approach has promising advantages such as mild working conditions, no inhibitor generation, minimal waste production, etc. However, there are still drawbacks such as long treatment time or low digestibility capacity which can be improved by utilization of microbial consortium. Filamentous fungi, cellulolytic and ligninolytic bacterial species were evaluated as promising degraders.

In this study, the construction of an efficient microbial community composed of strains was aimed. Therefore, some environmental factors including medium culture, medium pH, liquid:solid ratio, as well as cultivation method, were optimized for the enhancement of process efficacy.

MATERIALS AND METHODS

Microorganisms

Cellulolytic and ligninolytic bacteria were refreshed for 24 hrs in nutrient medium (NCAIM 0025); fungi strains *A. niger* NCAIM F.00632 was grown for 5 days on yeast extract peptone dextrose (YEPD) agar slants.

Optimization of microbial pretreatment

❑ *Effect of culture medium and pH*

Citrate buffer solution supplemented with mineral compounds and basal medium prepared with nutrient components were studied. The pHs of 4.5 and 6.5 were evaluated.

❑ *Effect of liquid:solid ratio*

Liquid:solid ratios ranging from 4:1 to 9:1 were tested.

❑ *Effect of cultivation method*

Co-culture of filamentous fungi *A. niger* F.00632 (FA) and lignocellulolytic bacterial co-culture *B. subtilis* B.01162 (A) and *P. putida* B.01522 (K*) were cultivated under suspended pretreatment (liquid:solid ratio of 9:1) or submerged pretreatment, in different cultivation orders.1

Analytical methods

- Weight loss: Using gravimetric analysis[1].
- Reducing sugar: Using Somoyi-nelson method[2].
- Enzymatic activities: Filter paper enzyme activity/total cellulase activity (FPase), carboxymethylcellulase/endo-glucanase (CMCase), xylanase were measured based on the activities of enzymes to hydrolyze proper substrates to reducing sugars[2,3].

RESULTS

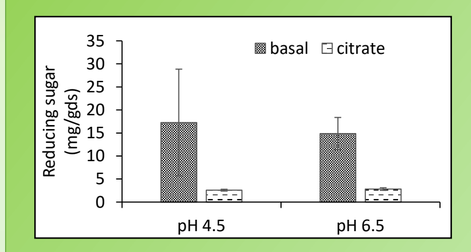

Figure 1. Effect of pH on fungal pretreatment under submerged condition

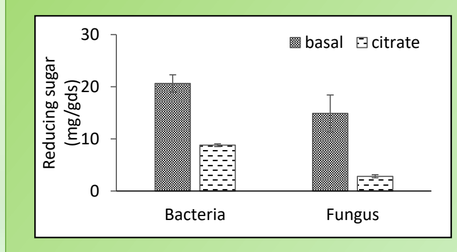

Figure 2. Effect of culture medium to pretreatment by fungus and bacterial co-culture

Effect of culture medium and pH

- Filamentous fungi can release reducing sugar yields of 6.67 and 5.27 fold-higher in pH 4.5 and pH 6.5 than those grown in citrate buffer, respectively. The supplements in culture medium can enhance fungal growth and degradation of biomass[4].
- pH values of 4.5 and 6.5 did not show any significant differences in lignocellulosic degradation efficiency.
- Bacterial co-culture prefers basal medium to citrate buffer with an increase of reducing sugar yield of 2.5 times higher.

Table 1. Enzyme production by bacterial and fungal species after 72 hour of biological pretreatment of lignocellulosic biomass

	Bacterial co-culture		Filamentous fungi	
	Citrate	Basal	Citrate	Basal
FPase	0.03 ± 0.01	0.02 ± 0.00	0.03 ± 0.01	0.04 ± 0.01
CMCase	0.18 ± 0.01	0.10 ± 0.00	0.48 ± 0.12	1.41 ± 0.52
Xylanase	0.60 ± 0.04	0.51 ± 0.01	6.15 ± 1.05	11.64 ± 0.01
Laccase	0.86 ± 0.01	4.32 ± 0.11	1.43 ± 0.40	1.11 ± 0.04

- Hydrolytic enzymes including total cellulase, endo-glucanase and xylanase produced by fungi cultivated in basal medium possessed significantly higher activities than those assayed from bacterial approach, approximately 2.7-10 times higher.
- On the other aspects, maximum laccase activity was observed in citrate buffer under bacterial co-culture cultivation.

RESULTS

Effect of liquid:solid ratio

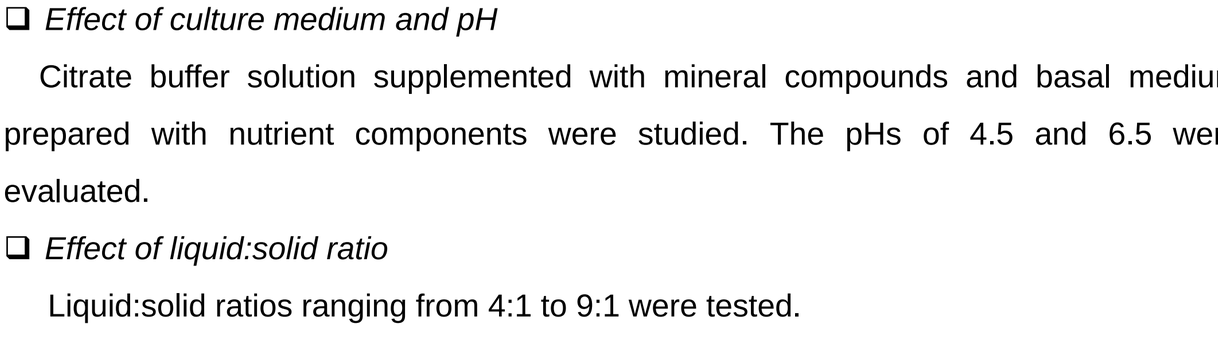

Figure 3. Effect of liquid:solid ratio on degradation efficiencies in bacterial and fungal pretreatment

- The liquid:solid ratio play a key role in pretreatment by filamentous fungi cultivation[5,6] and the ratio of 9:1 was accounted for the highest reducing sugar yield of 250 mg/gds after 72 hours of pretreatment.

Effect of cultivation method

Table 2. Experimental design for evaluation the effect of cultivation method

		Inoculation time	
	Denoted	**0 hr**	**24 hrs**
Suspended	I-A	Fungus	Bacteria
	II-A	Bacteria	Fungus
	III-A	Mixed culture	
Submerged	I-B	Fungus	Bacteria
	II-B	Bacteria	Fungus
	III-B	Mixed culture	

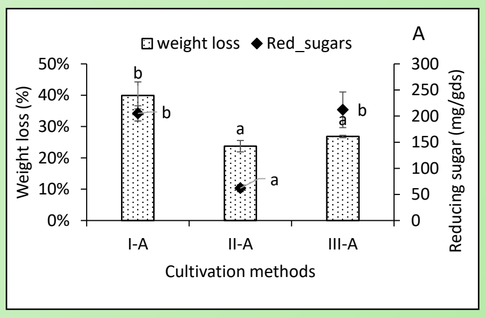

Figure 4. Effect of cultivation methods on degradation efficiency in suspended (A) and submerged (B) pretreatment

- In the suspended conditions, firstly-inoculated *A. niger* followed by bacterial co-culture 24 hrs later resulted in a high weight loss of 40% and 10 times increase of reducing sugar yield compared to the approach in which bacterial species inoculated firstly.
- Surmerged route showed insufficient degradation capacity under cultivation of microbial consortium.

Microbial consortium composed of filamentous fungi and cellulolytic-ligninolytic bacterial co-culture showed the sufficient degradation capacity when using basal medium at pH 6.5 as liquid phase, and an optimal liquid:solid ratio of 9:1 was utilized. The approaches in which firstly-inoculated A. niger followed by bacterial co-culture 24 hrs later or simultaneous cultivation in suspended conditions can be applied to enhance biological pretreatment efficiency.

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