Cost-effective production of *Trichoderma viride* lignocellulolytic enzyme cocktail by solid-state fermentation using wood biorefinery sludge: Effects of fermentation inhibitors and organic contaminants

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Abstract:
As the world hustles towards urbanization, Industrial waste management concepts, like circular economy, are considered as principles for eco-innovation which aims at a “zero waste” society and economy. In this context, biorefinery (BR) sludge, which is otherwise discharged as waste, can be contemplated as a valuable fermentation medium for the production of enzymes. The present study emphasizes on medium engineering for the cost-effective production of lignocellulolytic enzyme cocktail (Cavka *et al.*, 2013) by *Trichoderma viride*, through solid-state fermentation (SSF) where BR sludge was valorized as substrate, with the concurrent degradation of organic contaminants and fermentation inhibitors. The optimal conditions for enzymes production, such as cellulase (21 U/g), xylanase (84 U/g), laccase (20 U/g), lignin peroxidase (14 U/g) and aryl alcohol oxidase (116 U/g), coupled with contemporaneous degradation of organic contaminants and fermentation inhibitors was 192 h of incubation at 27°C, using a mixture of water and substrate in the ratio 1:1 (w/v), with 10% inoculum size. Further studies unveiled that, although the enrichment of the sludge with glucose, yeast extract and malt extract enhanced the lignocellulolytic enzyme cocktail by 5-6 folds, but the removal of organic contaminants and fermentation inhibitors remained unaltered. Additionally, the supplementation of 0.25 % rhamnolipids to the nutrient enriched sludge provided nearly 80–96% removal of phenol, pentachlorophenol, 2,4 dinitrophenol, benzo(a)pyrene and phenanthrene, along with 90-95% removal of furfural, 5-Hydroxymethylfurfural, Levulinic acid, Ferulic acid and Catechol.

The extensive testing of SSF has resulted in effective treatment of the toxic BR sludge (Singhania *et al.*, 2009) and the exploitation of the non-sterile conditions reduces the capital and operating cost. Innovation is the key to success and it is imperative to remain at par with the ever-changing demands of the industries. Thus, with better understanding of the scale-up of SSF and designs for commercialization, this fungal-assisted bioprocess system can be possible scaled-up, as the whole process proves to be eco-friendly and economically feasible.

Table 1: Production of lignocellulolytic enzyme cocktail and the concurrent degradation of organic contaminants and fermentation inhibitors.

	Control (CRB BR sludge)	**BR Sludge + GYM Media**	**BR Sludge + GYM* Media + 0.25% Surfactant**
Enzyme cocktail Production (U/g)			
Laccase	20	319	659
Xylanase	84	416	402
Cellulase	21	295	355
Lignin peroxidase	14	124	241
Aryl alcohol oxidase	116	811	1489
Organic Contaminants Removal (%)			
Pentachlorophenol	22	38	95

Phenanthrene	24	37	95
Benzo(a)pyrene	18	23	84
Phenol	96	95	96
2,4-dinitrophenol	38	58	95
Fermentation inhibitors removal (%)			
Furfural	95	93	95
HMF	94	92	95
Levulinic Acid	95	94	96
Ferulic Acid	61	63	93
Catechol	96	96	95

*GYM- Glucose, Yeast Extract and Malt Extract

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