A Study on Solid State Fermentations of Sugarcane Bagasse using Different Fungi

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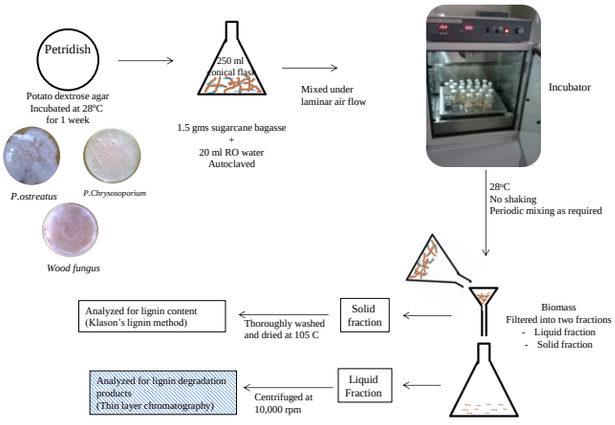
Introduction:

This research work focuses on microbial degradation of lignin in lignocellulosic biomass (sugarcane bagasse) by different fungal strains and study of their growth characteristics. A comparison of fungal growth characteristics, biomass solubilization and lignin degradation amongst the different fungi is presented.

The major constituents of sugarcane bagasse are cellulose (39-46% dry weight basis), hemicellulose (23-27%) and lignin (21-31%) [1]. Traditional pre-treatment methods for lignin breakdown require high energy inputs and usage of hazardous, harmful chemicals. In recent years biological pretreatment using fungi has received renewed attention which apart from causing selective degradation of lignin, also offers milder, eco-friendly process conditions. However this process takes more time than conventional methods. Considerable efforts are thus required to optimize the process parameters to reduce the time and increase the final yields of the product.

Solid state fermentations of sugarcane bagasse using different strains of fungi were carried out. The different fungi whose growth characteristics in the fermentations were studied are: *Phanerochaete chrysosporium* (1), *Pleurotus ostreatus*(2), a locally isolated fungus from wood (wood fungus) (3) and a mixed culture of *P. chrysosporium* and wood fungus (4). Depending on the fungi used, differences in solubilization, shrinkage and lignin degradation in the biomass were observed. Spectrophotometry was employed for analyzing the lignin content (Klason's method) [2].

Materials and methods:



Klason's Method [2,3]:

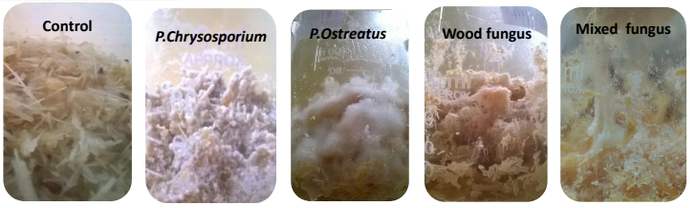
- To 0.25 g of oven dry sample, 5 ml of 72% H_2SO_4 is added and kept for 2 hours at 20 °C with occasional stirring.
- The acid is diluted to 3% conc. by adding 193 ml water and autoclaved at 121°C for 1 hour.
- The sample is filtered. The insoluble lignin is thoroughly washed with hot water and dried at 105°C till constant weight is achieved. This fraction is called acid insoluble lignin.
- The filtrate is analyzed for acid soluble lignin fraction by measuring the absorbance at 200nm in spectrophotometer.

Results and Discussions:

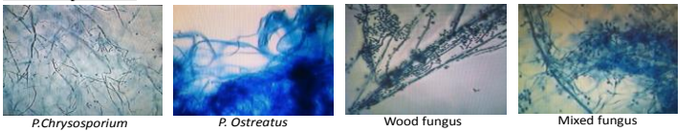
Sr. No	Microorganism	Age (days)	Growth characteristics on biomass	% Weight reduction of the biomass	% Lignin degradation*
1	*P.Chrysosporium*	20	- Abundant growth after 2-3 day lag phase - Periodic mixing aids cell growth - Softening and visible shrinkage of the biomass volume and significant generation of liquid fraction	32%	27%
2	*P. Ostreatus*	20	- Lower growth rate compared to P.Chrysosporium but abundant growth. - Periodic mixing aids cell growth - Biomass is not solubilized significantly in to liquid fraction - Small white colonies seen initially develop into an aggregated network along with the biomass	29%	23%
3	Wood fungus (Locally isolated fungus from wood)	20	- Long filaments (3-4 inch) prefer to grow aerially on the biomass surface. - Better penetration of the biomass by the filaments. - Filaments develop orange color over time (and biomass turns darker) - Prefers unmixed and relatively dry environment	27%	20%
4	Mixed fungus (*P.Chrysosporium* + Wood fungus)	20	- Hybrid growth characteristics of both the fungal species - Better penetration of the biomass by the filaments. - Visible shrinkage of the biomass volume and significant generation of liquid fraction	29%	27%

* % Lignin degradation = $\frac{(\%\ lignin\ in\ control - \%\ lignin\ in\ sample)}{(\%\ lignin\ in\ control)}$

Solid state fermentation:



Microscopic view:



Conclusion:

- Growth characteristics and lignin degradation of the sugarcane bagasse by the four fungal strains were studied.
- All strains showed abundant growth on biomass alone (No additional nutrients added).
- Wood fungus shows promising growth characteristics in terms of its ability to penetrate the biomass.
- *P. Chrysosporium* and mixed fungus showed the maximum biomass degradation, although selective degradation of lignin needs to be evaluated.

Ongoing and future work:

- Characterization of wood fungus.
- Comparison of the four fungal strains for selective degradation of lignin (by estimation of cellulose content).
- Optimization of process conditions for growth, selectivity and product formation.

Literature cited:

1. Larissa Canilha, Anuj Kumar Chandel et al., Bioconversion of Sugarcane Biomass into Ethanol: An Overview about Composition, Pretreatment Methods, Detoxification of Hydrolysates, Enzymatic Saccharification, and Ethanol Fermentation. Journal of Biomedicine and Biotechnology, Article ID 989572 (2012).
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3. Acid-insoluble lignin in wood and pulp, Reaffirmation of T 222 om-02, TAPPI Journal (2006).

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