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Biofuel production from agricultural residues

By

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B.Sc. (Microbiology) 2002 , M.Sc. (Microbiology) 2015
Faculty of Science – Benha University

Ph.D. THESIS

Submitted in Partial Fulfillment of the Requirements for the Degree of Ph. D. In Science (Microbiology)

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2022

2List of Abbreviations

A. fumigatus	*Aspergillus fumigatus*
A. flavus	*Aspergillus flavus*
A. neoellipticus	*Aspergillus neoellipticus*
A. niger	*Aspergillus niger*
AIC	Akaike Information Criterion
ARC	Agricultural Research Center
B. cereus	*Bacillus cereus*
BHT	Butylated hydroxytoluene
C. tropicalis	*Candida tropicalis*
C:N	Carbon : Nitrogen
CBP	Consolidated bioprocessing fermentation
CMC	Carboxy methyl Cellulose
CTAB	Cetyltrimethyl ammonium bromide
CYA	Czapek's yeast extract agar medium
DNS	3,5-dinitrosalicylic acid
Emim	1-ethyl-3-methylimidazolium acetate
FPase	Filter paper units
GC-MS	Gas chromatography–mass spectrometer
LSD	least significant differences

MEA	Malt extract agar medium
Mha	Million hectare
ML	Maximum-likelihood
MP	Maximum parsimony
MSM	Modified mineral salt medium
Mt/year	Million ton/year
NaClO	Sodium hypochlorite
NRRL	Northern Regional Research Laboratories
PDA	Potato-dextrose agar
PDA	Potato-dextrose agar
Rs	Rice straw
S. cerevisiae	*Saccharomyces cerevisiae*
Sacch %	Saccharification percentage
SmF	Submerged Fermentation
SSF	Simultaneous saccharification and fermentation
SWERI	Soil, Water and Environmental Research Institute
U/g RS	Units per gram dry rise straw

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6.Summary

Several fungal candidates were investigated for their cellulytic activities including two strains *Trichoderma viride* (NRRL1698) and *Aspergillus terreus* (NRRL260), isolates from soil at Agricultural Research Center, mangrove soil and compost samples.

Cellulytic activity of isolates and strains

All fungal candidates found to possess cellulytic activities using treated rice straw (RS) all over the 12 days of production and mostly achieved their maximum cellulytic activity at the end, of which strain NRRL1698 and isolates 516, 217, 504 and 517were the most promising fungal candidates.

Optimizing cellulase production

Cellulase production by any of the fungal candidates was subjected to successive optimization studies. The five fungal candidates 217, NRRL1698, 504, 516 and 517 achieved their maximum cellulytic production statistically after 5 days to be 7220U, 8124U), 7487U, 6203U and 7856U, at pH values of 5, 4, 4, 6 and 4, respectively. Exceptionally, both fungal candidates 504 and 517 achieved their maximum cellulytic production not only at pH 4 but also at pH 5, while 516 achieved its maximum cellulytic production at both pH 6 and 7.

The optimization of N source included studying the impact of two inorganic and two organic types on cellulase production. All fungal candidates achieved their maximum cellulytic activities statistically using organic nitrogen sources than inorganic ones, as isolates 217 and 517 recorded their best cellulytic activities using beef extract to be 7065U and 6842), strain NRRL1698 and isolate 516 recorded their best cellulytic activities using yeast extract to be 6499U and 8495U, while isolate 504 recorded its best cellulytic activities using either yeast or beef extracts to be 6293U and 6264U, respectively.

Saccharification

As both isolates 217 and 516 proved their superiority among other fungal candidates as efficient cellulase producers, their cellulases were tested for their efficiency in saccharifying treated rice straw (RS).

Optimizing saccharification in test tubes

Saccharification of RS was much better increasing by incubation period progressing on. As emphasized by statistical analysis the cellulases produced by isolate 217 maximum saccharification was better with pH 6 and 7 than with pH 4 at 40°C, while it was better with pH 5 than pH 6 and 7 at 50°C, proving that the cellulases preferred acidic pH at higher incubation temperature and neutral pH at lower temperature. On the other hand, the cellulase produced by isolate 516 was superior in saccharification results according to statistical analysis at pH 7 and temperature 50°C, followed by pH 6 at both temperatures 40°C and 50°C. Depending on those results, the cellulases produced isolate 516 proved to be more superior than that produced by isolate 217 in saccharification of treated RS.

Saccharification in flasks

The saccharification % of RS at 40°C / pH=6 fluctuated during incubation period achieving its highest result to be 72.19 % after 4 days and increased by 4.7 times compared to that in the test tube test. On the other hand, the saccharification % increased at the end of incubation period recording 89.11% that leveled up saccharification to 5.4 times compared to that achieved in previous test tube test.

Fermentation efficiency in bottles as affected by initial pH

Fermentation efficiency % increased parallel to fermentation period under each pH value, where it didn't exceed 13% up till 45 hr under both pH values 4 and 5, achieving their maximum after 48 hr to be 79.4 and 98.1 %, respectively. Fermentation efficiency % at pH=6 exceeded 50% after 30 hr and reached after 48 hr to be 99.2% where its ethanol yield was close to the ideal theoretical yield.

Rates of ethanol production in fermentation bottles

The production rate after 1 hr recorded 1.212, 1.013 and 3.001 g ethanol/bottle/hr declining afterwards it fluctuated to give occasional losses in ethanol produced where negative production rates appeared and finally achieving 2.358, 2.917 and 0.652 g ethanol/bottle/hr at the end of fermentation period, under pH values of 4, 5 and 6, respectively. It was an obvious increase in production rates under pH 4 and 5 nearly by 200% and 290%, respectively, comparing first hour and final 48 hr fermentation rates. Under pH 6 the first hour fermentation rate decreased by more than 78%, regardless to the accumulation final results.

Fermentation tests in fermenter

The fermentation efficiency in the first day achieved 64% and then decreased down till 4 hr after which it continued increasing up to 84% at 8 hr. In the second day an obvious decrease down to 63% followed by fluctuating increase achieving its maximum at 29 hr with a final fermentation efficiency of 100% after 48 hr.

Rate of ethanol production (g/fermenter/hr)

The rate of ethanol production spotted on the apparent loss in ethanol specifically after 2, 4, 24 27 30 and 48 hrs. Never the less, the fluctuation prolonged the accumulation period needed to achieve the 100% fermentation efficiency after 48 hr.

The comparison of fermentation efficiencies of reducing sugar by *S. cerevisiae* in both the 200ml fermentation bottles and 2L fermenter was crucial to emphasize the effect of fermentation volume and accordingly the effect of S. cerevisiae inoculum size on this process. Fermentation efficiency in fermenter was higher than that in fermentation bottle under the same fermentation conditions. This made the ethanol harvesting during fermentation period more feasible from fermenter than bottles, if it was intended to be used.

The genetic identification of isolate 516

Sequences of fungal isolate 516 designated as Am1 in this study were assembled using DNASTAR computer package (version 5.05). Assembled sequence of isolate Am1 was uploaded to GenBank as OM760501. The closely similar sequences to Aspergillus: section Fumigati including sequences of type and ex-type species were downloaded from GenBank. The isolate AM 1 occupied the same branch as *Aspergillus neoellipticus* ATCC 16903 (type strain) with 100% (562/562) similarity between both species. As a result, this isolate was identified as *Aspergillus neoellipticus*.

GC mass results

Fermentation final sample was assessed qualitatively by GC-MS analysis, as the constituents in the mass spectrum fragmentation pattern obtained by electron ionization (EI) were compared with those stored in Wiley and NIST Mass Spectral Library data. Cation fragments appearing at specific m/z segmented from the main parent molecule by losing specific part. The comparative spectrum proved that the final product was ethyl alcohol.