Fungal Solubilisation and Subsequent Microbial Methanation of Coal Processing Wastes

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Research Article

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Abstract

Large quantities of rejects from coal processing plants are currently disposed of as waste piles or in ponds and rivers, resulting in environmental concerns including pollution of rivers and ground and surface water contamination. This work investigates for the first time, a two-stage microbial process for converting coal processing wastes to methane, involving (1) fungal solubilisation of coal rejects and (2) microbial methanation of the solubilised products. Phanerochaete chrysosporium, Trichoderma viride and Neurospora discreta were screened for their ability to solubilise coal rejects. N. discreta was found to be the most suitable candidate based on the extent of bio-solubilisation, laccase activity, and reversed-phase high performance liquid chromatography (RP-HPLC) analysis. Bio-methanation of fungal-solubilised coal rejects was carried out in mesophilic anaerobic reactors with no additional carbon source, using inoculum from an anaerobic food digester. Coal rejects solubilised by N. discreta produced 3 to 6-fold higher methane compared to rejects solubilised by the other two fungi. No methane was produced from untreated coal rejects, demonstrating the importance of the fungal solubilisation stage. A total of 4.1 mmol methane was generated per gram of carbon in 15 days from N. discreta-solubilised coal rejects. This process offers a timely, environment-friendly, and sustainable solution for treatment of coal rejects and the generation of value-added products such as methane and volatile fatty acids.

Introduction

Coal remains one of the most significant energy resources around the world with a global consumption of nearly 8000 Mt per year [1]. Continuing to meet this demand despite steadily depleting deposits of high-rank coal has led to the mining of low-rank coals such as sub-bituminous coal and lignite. China and India, two of the largest coal producing countries, have abundant reserves of low-rank coals. In the United States, another significant coal producer, the trend has shifted in recent years towards the mining of sub-bituminous coal.

Low-rank coals have high ash and moisture content and low thermal efficiencies compared to high-rank coals such as anthracite and therefore need to be subjected to coal beneficiation or upgradation to reduce ash content before being used for power generation [2–4]. However, as the process of separating ash from coal is particularly challenging for low-rank coals, nearly 30–40% of coal is rejected in coal processing plants, resulting in millions of tonnes of coal waste every year [3, 5–7]. Depending on the beneficiation process, dry coal rejects are typically disposed of as coal waste piles while coal reject slurries are discarded in rivers (especially in India) or within embankments or ponds [8]. These disposal methods have led to serious environmental issues including pollution of rivers, ground and surface water contamination from reject area leachate, and fugitive emission of dust [5, 7, 9].

Coal rejects typically contain more than 50–60% ash but also contain up to 15% carbon and other combustibles that can potentially be utilised [10]. Recent research has explored the utilisation of coal rejects in fluidised bed combustion [9] and the recovery of clean coal from washery rejects using physical
and chemical methods [5, 10]. However, high inputs of energy, the need for high-strength chemicals and low recoveries from these processes currently render these methods largely non-viable.

Can a biological process for treating coal rejects offer a sustainable and environment-friendly solution to these challenges?

Although studies on biodegradation of coal wastes are limited, filamentous fungi such as *Trichoderma viride* and *Phanerochaete chrysosporium* and certain aerobic bacteria have been shown to degrade low-rank coals such as lignite [11–17]. These microorganisms contain multiple ligninolytic and other oxidative and reductive enzymes that carry out the depolymerisation and bio-solubilisation of the coal structure, which is similar to that of lignin for low-rank coals [12]. Studies with lignite have shown the degradation of the coal matrix to lower molecular weight aromatic and aliphatic compounds that could potentially be converted to value-added products [16, 18, 19].

A different set of studies has explored the microbial generation of methane from coal, arising from the recent understanding of the role of microorganisms in coalbed methane generation – originally considered to be a purely thermogenic process [20]. Microbial methane production from sub-bituminous coal and lignite has been demonstrated at lab-scale, although this is a relatively slow process taking more than 60–70 days and even up a few hundred days in some cases [21–23].

These bio-solubilisation and bio-methanation studies independently demonstrate that low-rank coal can be microbially converted to either liquid products or to methane, although the significantly long process durations remain a challenge in the case of methane production. A gap exists in evaluating a combined approach of bio-solubilisation and bio-methanation, to improve the digestibility of the coal matrix for methane production. Furthermore, till date no similar studies have been reported on coal processing wastes or coal rejects. It is useful to note the differences between coal rejects and low-rank coal as potential substrates for microorganisms. Coal rejects have significantly higher ash content and lower carbon content compared to low-rank coal. Lignite for instance, contains about 60–70% carbon [20] while coal rejects contain less than 20% carbon. The structure of coal rejects is also likely to be less recalcitrant than that of coal, making it easier to degrade. This, coupled with the fact that coal rejects are currently a wasted resource, makes coal rejects a promising substrate for microbial methane production.

The present work is based on the hypothesis that coal rejects can be converted to methane using a two-stage biological process: (1) fungal solubilisation of coal rejects to produce simpler, water-soluble degradation products and (2) bio-methanation of the solubilised products using anaerobic microorganisms. Considering the environmental hazards posed by inappropriate disposal of these rejects, and the large quantities in which they are produced, this process offers a timely, sustainable, and environment-friendly solution not only for treatment of coal rejects, but also for extracting a valuable fuel in the form of methane.

**Materials And Methods**
Coal Rejects

Samples of coal-washery rejects sourced from Talcher coal mines, India, were kindly supplied by Ardee Hi-Tech Pvt Ltd, Visakhapatnam, India. The particle size and minimum ash content of the coal rejects were 0.2 mm and 75% respectively.

Fungal solubilisation of coal rejects

Three fungal species were screened for their ability to solubilise the coal rejects. *Phanerochaete chrysosporium* (NCIM 1197) and *Trichoderma viride* (NCIM 1060) were obtained from National Collection of Industrial Microorganisms, Pune, India. These two fungi were selected for their reported ability to degrade low-rank coal [12, 14, 17]. The third fungus, *Neurospora discreta* was previously isolated from a Subabul wood tree and was selected for its ability to produce ligninolytic enzymes and degrade lignin [24, 25]. All fungi were sub-cultured on potato dextrose agar (PDA) plates and at 2-8°C until further use.

Fungal solubilisation of coal rejects was carried out as submerged fermentation in 250 mL Erlenmeyer flasks containing 100 mL Vogel’s minimal medium [26] with 1 g sucrose and 1 g coal rejects. After sterilisation and cooling, 0.1% biotin solution was added to each flask, and the flasks were inoculated in triplicate with a spore suspension of each fungal species. To prepare the spore suspension, cells were scraped from the agar plates and filtered through a muslin cloth and the spore suspension obtained was added to each flask to get a final concentration of 0.2 million spores per mL. All flasks were then incubated in a shaker incubator at 30°C and 100 rpm for 14 days. Un-inoculated coal rejects in Vogel’s medium were set up as controls.

Analysis of solubilised products, enzyme activity, protein content and dry weight

Liquid samples were taken from each flask at regular intervals, centrifuged to remove solids and analysed using RP-HPLC on a C-18 column, using a mixture of acetic acid and acetonitrile as the mobile phase using the method described elsewhere [24]. Alkali lignin (low sulphonate Kraft lignin, Sigma Aldrich) was used as a reference standard. Controls (coal rejects without fungal treatment) and a media blanks were also run using the same method.

Liquid supernatant obtained after centrifugation of samples from each flask was analysed for laccase activity based on oxidation kinetics of ABTS. Absorbance of the blue-green radical formed by the enzymatic oxidation of ABTS was measured at 420 nm and enzyme activity was calculated as the amount of enzyme forming 1 µM.min\(^{-1}\) of product, using an extinction coefficient (\(\varepsilon_{420}\)) of 36000 L.mol.cm\(^{-1}\) [25].

Protein content in the solid fraction was used as an indirect measure of cell growth. For this, a known mass of the solid fraction was subjected to protein extraction by incubating with Radio-Immunoprecipitation Assay (RIPA) lysis buffer containing 1mM phenyl methyl sulphonyl fluoride (PMSF)
(both from Sigma Aldrich) for one hour at room temperature with manual glass bead vortexing every 15
minutes. 10 ml of buffer was used per gram of solid. The lysate was then centrifuged at 2500 x g for 5
minutes and the protein content in the supernatant was estimated using the Folin-Lowry method [27].

Dry weight of the residual coal was obtained after drying the solid fraction at 103.5°C in an oven until
constant weight was achieved.

**Bio-methanation of fungal-solubilised coal rejects**

**Batch reactor set-up**

A schematic representing the fungal solubilisation and bio-methanation experiments is shown in Fig. 1.
Batch bio-methanation studies were carried out in 250 mL serum bottles using the fungal-solubilised coal
samples. In the figure and description below, the letters N, P, T denote coal rejects subjected to bio-
solubilisation by *N. discreta*, *P. chrysosporium*, *T. viride* respectively and C denotes the control (coal
rejects without fungal treatment). Each reactor contained 40% by volume of the bio-solubilised coal and
45% modified Barker's medium [28]. The medium contained 20 g.L⁻¹ CaCO₃, 1.0 g.L⁻¹ NH₄Cl, 0.4 g.L⁻¹
and K₂HPO₄·3H₂O but with no additional carbon source. All reactors were purged with nitrogen for 5–7
minutes with a long needle while simultaneously boiling the medium to remove oxygen and then sealed
with rubber septa and aluminium crimp seals to maintain an anaerobic environment. After autoclaving
and cooling, sterile 0.5mM Na₂S was added. Each reactor was then inoculated with 15% inoculum from a
mesophilic anaerobic digester for food waste, kindly supplied by BITS Pilani, Goa campus. Water was
added to the control in place of the inoculum. All reactors were incubated at 37°C. Methane concentration
in the headspace and volatile fatty acids VFA in the liquid samples were analysed as described below.

**Determination of volumetric methane production**

To determine the volume of methane produced in coal rejects solubilised by *N. discreta* (N-1, Fig. 1), the
reactor was sealed using a rubber stopper with a tube to allow the headspace gas to exit (instead of the
crimp). The gas passed through a solution of 0.1 M calcium hydroxide solution to strip CO₂ and into an
inverted measuring cylinder filled with water in a water trough. The volume of methane gas was
determining by the volume of water displaced in the measuring cylinder.

**Effect of media addition**

In a separate study (N-2, Fig. 1), once the methane gas production slowed down in the batch reactors,
45% degassed Barker's medium was added to 55% of the broth from the batch reactor (N) under
anaerobic conditions. As before, no additional carbon source was added. Liquid samples were withdrawn
anaerobically for VFA analysis, and the headspace gas was analysed for methane as described below.

**Determination of methane gas concentration and VFA**

Methane gas in the headspace was measured using a portable biogas analyser (BIOGAS 5000, Geotech,
India), connected to a needle to pierce the rubber septa.
Liquid samples from the anaerobic reactors were centrifuged at 10,000 x g for 10 minutes and the supernatant was put through a 3-point titration for pH 5.0, 4.3 and 4.0. Total VFA was calculated according to the following formula [29, 30]:

\[
Total\ VFA\ (mg.\ L^{-1}) = \left[131.340 \times (V_{pH4.0} - V_{pH5.0}) \times \frac{N_{H_2SO_4}}{V_s}\right] - \left[3.08 \times V_{pH4.3} \times \frac{N_{H_2SO_4}}{V_s} \times 1000\right] - 10.9
\]

In the above formula, \(V_{pH4.0}\), \(V_{pH4.3}\) and \(V_{pH5.0}\) are the volumes (in mL) of acid added until pH of 4.0, 4.3 and 5.0 is achieved respectively. \(V_s\) is the volume of the titration sample in mL and \(N_{H_2SO_4}\) is the normality of sulphuric acid.

**Results And Discussion**

**Screening of fungal species for bio-solubilisation**

**Extent of bio-solubilisation and laccase activity**

The protein content in the solid biomass was similar for all three fungal species, indicating similar cell growth (Fig. 2). However, the mass of residual coal rejects varied based on the fungus indicating a difference in the extent to which the solid coal was solubilised in each case. At the end of 14 days, *N. discreta* resulted in a 55% reduction in the mass of coal rejects, which was the highest amongst the three species. *T. viride* resulted in the least reduction of approximately 25%.

This trend is further confirmed by the activity of laccase, which was the highest in the case of *N. discreta* followed by *P. chrysosporium* which showed significantly lower activity (Fig. 2). Laccases are one of the primary groups of enzymes responsible for de-polymerisation and bio-solubilisation of coal, owing to their low specificity and ability to break down both phenolic and non-phenolic structures [18, 31]. Extracellular laccases have been reported in all three fungi tested [25, 32, 33], however some studies have indicated intracellular, membrane-associated laccases in *T. viride* [34]. This could be one of the factors contributing to the absence of laccase activity in the *T. viride* samples. It is also likely that other ligninolytic enzymes were responsible for fungal solubilisation. However, the positive correlation between laccase activity and extent of fungal solubilisation in each case indicates the laccase played a significant role in the solubilisation of coal rejects.

**Analysis of bio-solubilisation products**

Bio-solubilisation of coal has been shown to occur via the breakdown of the hydrophobic coal matrix into simpler, water soluble (“liquified”) products [35, 36]. In the present study, fungal bio-solubilisation of coal rejects resulted in the production of polar degradation products as confirmed by RP-HPLC chromatograms of the liquid samples (Fig. 3). Owing to the structural similarities between lignite and lignin [12], it can be expected that solubilisation of coal would result in products similar to soluble lignin. Therefore, water-soluble alkali lignin was used as the reference standard.
Each fungal species used for bio-solubilisation produced a different profile of degradation products. As bio-solubilisation progressed from day 7 to 14, coal rejects treated with *N. discreta* and *P. chrysosporium* showed a decrease in product heterogeneity (number of peaks) and a slight increase in polarity (based on retention time) (Fig. 3a, b, d, e). Treatment with *T. viride* resulted in no significant peaks on day 7 (Fig. 3c), indicating a slower degradation compared to the other two cases.

On day 14, coal rejects treated with *N. discreta* produced a single larger peak at a retention time (RT) close to 2.6 minutes (Fig. 3d), indicating the presence of a highly polar product similar to the soluble lignin standard (Fig. 3g). Solubilisation by *P. chrysosporium* and *T. viride* resulted in multiple smaller peaks (Fig. 3e, f). The coal control (without fungal treatment) sample consistently had a few small peaks, all below an intensity of 5 mAU.

A comparison of the areas under the curve (AUC) corroborates the observation from dry weights and enzyme activities that *N. discreta* resulted in the highest extent of bio-solubilisation, and *T. viride* the lowest (Fig. 4). In all cases the total AUC increased from day 7 to day 14 indicating the progress of bio-solubilisation with time.

### Production of methane and VFA

In the batch bio-methanation studies, methane production from coal rejects treated with *N. discreta* (reactor N, Fig. 1) increased steadily till day 15, after which the rate of increase slowed down (Fig. 5). By day 23, the reactor headspace contained 60% methane which was six-fold higher than in reactor T and three-fold higher than in reactor P. Coal rejects without fungal treatment did not produce any methane in the time period tested. This can be compared to studies reported with low-rank coal wherein methane production did not commence until after approximately 60 days [21–23].

Figure 5 in conjunction with Fig. 4, highlights the importance of the first stage in methane production and shows a positive effect of the extent of fungal solubilisation of coal rejects on methane production. This can be explained by the fact that the products of bio-solubilisation are simpler structures that are easier to utilise by methanogens. Moreover, the polar nature of these products (as seen from the RP-HPLC chromatograms) significantly improves accessibility to the microorganisms compared to the highly hydrophobic coal particles.

VFA at harvest showed the opposite trend to methane production with 3-fold higher VFA production seen in coal rejects treated with *T. viride* compared to *N. discreta* as seen (Fig. 4A). VFAs are intermediate products in the methanogenic pathway, arising from the hydrolysis of the substrate and serving as precursors to methane formation. Therefore, a high concentration of methane, as in the case of *N. discreta* and a relatively low residual VFA content in the reactor indicates the conversion of VFA to methane. Solubilisation by *P. chrysosporium* resulted in lower methane but higher VFA compared to *N. discreta*.

Interestingly, the high VFA concentration in *T. viride*-treated samples indicates that the anaerobic consortium was able to metabolise the degraded and solubilised coal products to some extent, although
this did not translate to methane production in the given time scale. Longer periods of solubilisation and bio-methanation could increase methane production in these cases.

As discussed previously, the methane production in *N. discreta* slowed down between days 15 and 23 increasing by only 2%. However, addition of fresh Barker’s medium to the *N. discreta*-treated sample in the second stage (reactor N-2, Fig. 1) resumed methane production, which built up to over 35% in 10 days. This indicated that the slowdown in methane production in the first stage was not due to depletion of the carbon source (coal rejects) but due to depletion of other nutrients or a build-up of inhibitory by-products. It is to be noted that there was no residual methane on day 0 in the headspace as the substrate, culture and fresh medium were transferred to a new reactor. However, residual VFA from the previous culture can still be seen in N-2 on day 0 and correlated well with the extent of dilution with fresh medium. In N-2, VFA dropped steadily with time reaching a value below 5 mg/L on day 10 once again confirming the conversion of VFA to methane.

From reactor N-1 (Fig. 1), 0.82 mmol (20 mL) of methane was produced per gram of coal rejects in 15 days. Direct biogenic methane production from low-rank coal has been reported at much lower levels starting at 14–16 µmol per g of coal in 70 days, to approximately 0.2 mmol per gram in 63 days [22]. Wang et al [37] found that pre-treating lignite with pre-acclimatised aerobic sludge bacteria for 28 days followed by anaerobic digestion resulted in nearly 0.2 mmol of methane per gram of coal which was thrice the amount produced without pre-treatment. Considering the differences in carbon content between lignite and coal rejects, a better comparison would be in terms of methane per gram of carbon. At an average value of 65% carbon in lignite, the highest methane production reported so far is 0.3 mmol per gram of carbon [22, 37] which is significantly lower than the 4.1 mmol of methane per gram of carbon observed in the present study.

**Conclusion**

This work demonstrates a two-stage conversion of coal rejects to methane for the first time, involving fungal solubilisation followed by microbial methanation. Fungal solubilisation of coal rejects resulted in highly polar degradation products as analysed by RP-HPLC. Of the fungal species tested, *N. discreta* was found to be the most suitable candidate as it resulted in the highest extent of bio-solubilisation and consequently the highest amount of methane production. Up to 60% methane was produced from coal rejects treated with *N. discreta* with a total of 4.1 mmol methane per gram of carbon in 15 days. This is more than ten-fold higher than the methane production reported from low rank coals such as lignite. This two-stage process offers an environment-friendly solution for the conversion of coal rejects to methane. This process can also be extended to the upgradation of low-rank coals to avoid the use of high temperatures and pressures and generation of harmful by-products and gases. Optimisation of process conditions at the bio-methanation stage can lead to further improvement in methane yields. An analysis of individual VFAs produced can help identify other value-added products from coal rejects.

**Declarations**
Acknowledgements

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Conflicts of Interest

The authors have no conflicts of interest to declare.

Availability of data

Data used during the present study can be requested from the corresponding author.

Author contributions

AA conceived and designed the experiments and wrote the manuscript. AS executed the experiments and collected data.

Ethics approval

Not applicable

Consent to participate

Not applicable

Consent for publication

Not applicable

References

1. IEA. (2020) Coal 2020: Analysis and forecast to 2025 International Energy Agency Report.

2. Duzyol, S. and Sensogut, C. (2018) Investigation of the Thermal Improvement and the Kinetic Analysis of the Enriched Coal. J. Combust. 2018, 1–10.

3. Zhao, Y., Yang, X., Luo, Z., Duan, C. and Song, S. (2014) Progress in developments of dry coal beneficiation. Int. J. Coal Sci. Technol. 1, 103–112.
4. Umar, D. and Daulay, B. (2011) Improvement of low rank coal properties by various upgrading processes. Indones. Min. J. 14, 17–29.

5. Lingam, R. K., Suresh, A., Dash, P. S., Kumar, S. and Ray, T. (2016) Upgrading Coal Washery Rejects Through Caustic-acid Leaching Upgrading Coal Washery Rejects Through Caustic-acid Leaching. Miner. Process. Extr. Metall. Rev., Taylor & Francis 37, 69–72.

6. Gillenwater, L. E. and Gillenwater, B. L. E. (1951) Coal washery wastes in West Virginia. Sewage Ind. Waste. 23, 869–874.

7. Chugh, Y. P. and Behum, P. T. (2014) Coal waste management practices in the USA: an overview. Int. J. Coal Sci. Technol. 1, 163–176.

8. Behum, P. T., Chugh, Y. P. and Lefticariu, L. (2018) Management of coal processing wastes: studies on an alternate technology for control of sulfate and chloride discharge. Int. J. Coal Sci. Technol., China Coal Society 5, 54–63.

9. MoEF. (2010) Environmental Impact Assessment Guidance Manual for Coal Washeries, Ministry of Environment and Forests, Govt of India.

10. Yu, Y., Li, Z., Zhang, N. and Qu, J. (2020) Deep recovery study for coking coal washery rejects using a comprehensive process. Energy Sources, Part A Recover. Util. Environ. Eff., Taylor & Francis 1–13.

11. Opara, a., Adams, D. J., Free, M. L., McLennan, J. and Hamilton, J. (2012) Microbial production of methane and carbon dioxide from lignite, bituminous coal, and coal waste materials. Int. J. Coal Geol. 96–97, 1–8.

12. Sekhohola, L. M., Igbinigie, E. E. and Cowan, A. K. (2013) Biological degradation and solubilisation of coal. Biodegradation 24, 305–18.

13. Manoj, B. (2013) Bio-demineralization of Indian Bituminous Coal by Aspergillus niger and characterization of the products 8, 49–54.

14. Silva-Stenico, M. E., Vengadajellum, C. J., Janjua, H. A., Harrison, S. T. L., Burton, S. G. and Cowan, D. A. (2007) Degradation of low rank coal by Trichoderma atroviride ES11. J. Ind. Microbiol. Biotechnol. 34, 625–31.

15. Denizli, A., Sakintuna, B., Taralp, A. and Yu, Y. (2003) Bio-Liquefaction / Solubilization of Low-Rank Turkish Lignites and Characterization of the Products. Energy and Fuels 17, 1068–1074.

16. Kang, H., Liu, X., Zhang, Y. and Zhao, S. (2021) Environmental Effects Bacteria solubilization of shenmu lignite: influence of surfactants and characterization of the biosolubilization products. Energy Sources, Part A Recover. Util. Environ. Eff., Taylor & Francis 43, 1162–1180.
17. R. C. Tripathi, V. K. Jain, P. S. M. T., Tripathi, R., Jain, V. and Tripathi, P. (2009) Fungal Biosolubilization of Neyveli Lignite into Humic Acid. Energy Sources, Part A Recover. Util. Environ. Eff. 32, 72–82.

18. Kwiatos, N., Krzepkowska, M. J., Strzelecki, B. and Bielecki, S. (2018) Improvement of efficiency of brown coal biosolubilization by novel recombinant Fusarium oxysporum laccase. AMB Express, Springer Berlin Heidelberg 8, 1–9.

19. Crawford, D. L. and Nielsen, E. P. (1995) Biotransformation of coal substructure model compounds by microbial enzymes. Appl. Biochem. Biotechnol. 54.

20. Strapoc, D., Mastalerz, M., Dawson, K., Macalady, J., Callaghan, A. V, Wawrak, B., Turich, C. and Ashby, M. (2011) Biogeochemistry of Microbial Coal-Bed Methane. Annu. Rev. Earth Planet. Sci. 39, 617–56.

21. Su, X., Zhao, W. and Xia, D. (2018) The diversity of hydrogen - producing bacteria and methanogens within an in situ coal seam. Biotechnol. Biofuels, BioMed Central 11, 1–18.

22. Gupta, P. and Gupta, A. (2014) Biogas production from coal via anaerobic fermentation. Fuel, Elsevier Ltd 118, 238–242.

23. Gupta, A. and Birendra, K. (2000) Biogasification of coal using different sources of micro-organisms. Fuel 79, 103–105.

24. Pamidipati, S. and Ahmed, A. (2017) Degradation of Lignin in Agricultural Residues by locally Isolated Fungus Neurospora discreta. Appl. Biochem. Biotechnol. 181, 1561–1572.

25. Pamidipati, S. and Ahmed, A. (2020) A first report on competitive inhibition of laccase enzyme by lignin degradation intermediates. Folia Microbiol. (Praha),, Folia Microbiologica 65, 431–437.

26. Vogel, H. J. (1964) Distribution of Lysine Pathways Among Fungi: Evolutionary Implications. Am. Nat. XCVIII, 435–446.

27. Lowry, O, H., Rosebrough, N, J., Randall, R. J. and Lewis, A. (1951) Protein measurement with the folin phenol reagent. J. Biol. Chem. 193, 265–275.

28. Baresi, L., Mah, R. A., Ward, D. M. and Kaplan, I. R. (1978) Methanogenesis from Acetate: Enrichment Studies. App 36, 186–197.

29. Buchauer, K. (1998) A comparison of two simple titration procedures to determine volatile fatty acids in influents to waste-water and sludge treatment processes. WAtier SA 24, 49–56.

30. Drosg, B. (2013) Process monitoring in biogas plants, IEA Bioenergy.

31. Toshiaki Kabe, Atsushi Ishihara, Eika Weihua Qian, I Putu Sutrisna, Y. K. (2004) Microbial Depolymerization of Coal. In Studies in Surface Science and Catalysis, pp 303–314, Elsevier.
32. Srinivasan, C., Souza, T. M. D. and Boominathan, K. (1995) Demonstration of Laccase in the White Rot Basidiomycete Phanerochaete chrysosporium BKM-F1767. Appl. Environ. Microbiol. 61, 4274–4277.

33. Smoleňová, E., Pokorný, R., Kaliňák, M., Liptaj, T., Šimkovič, M. and Varečka, Ľ. (2020) Degradation of low-rank coal excavated from coal-mine Záhorie by filamentous fungi 13, 14–22.

34. Approach, A. B. (2013) Structural and Phylogenetic Analysis of Laccases from Trichoderma: A Bioinformatic Approach 8.

35. Strzelecki, B. and Kwiatos, N. Effect of coal pretreatment on brown coal biosolubilisation by Fusarium oxysporum 1101.

36. Webb, H. K., Arnott, J., Crawford, R. J. and Ivanova, E. P. (2013) Plastic Degradation and Its Environmental Implications with Special Reference to Poly(ethylene terephthalate). Polymers (Basel). 1–18.

37. Wang, B., Tai, C., Wu, L., Chen, L., Liu, J., Hu, B. and Song, D. (2017) Methane production from lignite through the combined effects of exogenous aerobic and anaerobic micro flora. Int. J. Coal Geol. 173, 84–93.

**Figures**
Figure 1

Schematic of fungal solubilisation and bio-methanation studies. N', P', T' represent fungal solubilisation by N. discreta, P. chrysosporium, T. viride respectively and C' represents the control. N, P, T, C represent bio-methanation of the coal rejects treated with N. discreta, P. chrysosporium and T. viride respectively and C represents untreated coal rejects. N-1 was set up to measure the volumetric methane production and N-2 was sub-cultured from N by adding fresh Barker’s medium.
Figure 2

Mass of coal rejects before and after bio-solubilisation and protein content are depicted by bars and laccase activity is represented by the filled circles.
Figure 3

RP-HPLC chromatograms of liquid samples post fungal treatment of coal rejects. (a) N. discreta day 7 (b) P. chrysosporium day 7 (c) T. viride day 7 (d) N. discreta day 14 (e) P. chrysosporium day 14 (f) T. viride day 14 (g) Alkali lignin standard (h) Coal control (i) Media blank
Figure 4

Total area under the curve (AUC) calculated from RP-HPLC chromatograms of liquid samples after treatment with N. discreta, P. chrysosporium and T. viride. The control contains un-inoculated coal rejects in media.
Figure 5

a Methane and VFA production from coal rejects treated with different fungi as a function of time b Methane and VFA production after addition of fresh Barker’s medium (reactor N-2)