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The effect of the particulate phase on coal biosolubilisation mediated by *Trichoderma atroviride* in a slurry bioreactor

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ARTICLE INFO

Article history:
Received 3 January 2007
Received in revised form 1 June 2007
Accepted 20 June 2007

Keywords:
Trichoderma atroviride (ES 11)
Low rank coal
Particle size fraction
Coal loading
Coal biosolubilisation

ABSTRACT

Low rank coal is currently under-utilised because of its low calorific value and high moisture and sulphur content. Its solubilisation by both bacterial and fungal cultures has been reported, the latter more commonly. Coal biosolubilisation processes have potential to convert low rank coal to either a clean, cost-effective energy source or complex aromatic compounds for biocatalytic conversion to value-added products. This can lead to an increased utilisation of low rank coal. In this study, the key variables of the slurry that affect biosolubilisation of low rank coal by *Trichoderma atroviride* in submerged culture were investigated. Results showed that the key operating variables that influence coal biosolubilisation in the slurry bioreactor are coal loading and particle size affecting available surface area. These factors affect the surface area available for coal biosolubilisation. The optimum coal loading occurred between 5 and 10% (w/v); an increase above this optimum led to inhibition of the fungal culture of *T. atroviride* (ES11) by fragmentation of the fungal mycelium. A decrease in particle size fraction led to an increase in the degree of coal solubilisation. Coal biosolubilisation was shown to increase 4-fold when particle size was decreased from 600–850 μm to 150–300 μm. A 28% biosolubilisation of coal of 150–300 μm, characterised by a surface specific area of 2.17 $cm^2\ g^{-1}$, was measured as coal weight loss over 14 days at solids loading at 5%. This can be compared with a 7.8% coal weight loss at 600–850 μm diameters (0.54 $cm^2\ g^{-1}$). Soluble phenolic compounds are not a significant product of the coal biosolubilisation process. The change in pH observed in the presence of both coal and fungi was independent of coal loading and was not directly related to the extent of coal solubilisation. While soluble intermediates were observed as total organic, further metabolism resulted in complete oxidation of a significant fraction of the coal to CO_2.

1. Introduction

Fossil fuels remain the primary source of energy, with renewable energy sources providing an increasing, but small, contribution to the world's energy needs. Coal represents one of the world's most abundant fossil energy resources. The world's coal reserves are estimated at 1.53×10^{20} Btu or 71.4% of the world fossil fuel resource [1]. Thermal and chemical processes currently dominate conversion of coal to liquid and gaseous energy products. These generally require extreme conditions of temperature, pressure and chemical environment; hence efficient processing is limited to coals of high calorific value [2]. Coal is used for electricity generation, generation of industrial steam, transportation, residential heating, and conversion to liquid fuels. Coke (fixed carbon residue) from carbonisation of coal is used in metallurgical

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doi:10.1016/j.fuproc.2007.06.019

fabrication processes.Low rank coal is not used significantly in the above-mentioned processes through current coal conversion technologies owing to its low calorific value and high moisture and sulphur content. However, the reserves of low rank coal are substantial [2]. World reserves of low rank coal in 2002 were 47% of the total recoverable reserves of coal, while total low rank coal production in 2001 was approximately 28% of the total production of coal worldwide [3]. Microbial solubilisation of low rank coal at ambient temperature and pressure has been demonstrated [4–6] and reported to yield useful oxidised products [7]. These products have potential use as substrates in biotransformation processes for the production of value added compounds such as antioxidants [7]. The remaining unsolubilised coal fractions have been postulated to be enriched in aliphatic compounds with a low water and ash content [8]. The residual unsolubilised coal is characterised by a higher heating value. This can then be used as a clean and cost effective energy source [8].

Coal biosolubilisation has been determined qualitatively by the colouration of the supernatant measured by absorbance at 450 nm [3,9–12]. The liquid product from coal biosolubilisation includes humic and fulvic acids which impart a dark-brown colour and display a maximum absorbance at 450 nm.

The biosolubilisation of coal necessitates use of either a packed bed or slurry process to provide contact between the microbial phase and its enzyme products and coal. Particle size fraction influence the performance of both systems while coal loading is expected to impact the slurry process.

The literature presents a contradiction on the effect of particle size on coal biosolubilisation. Cohen et al. [9] observed that a decrease in particle size across the range 250 to 105 mm resulted in a higher degree of biosolubilisation when pre-treated coal was incubated in cell-free culture. This is in agreement with increased surface area and surface-based reaction. Conversely, Gokacy et al. [12] observed that an increase in particle size across the range 1500 to 2000 μm led to a higher degree of biosolubilisation. Earlier research on coal biosolubilisation was carried out across the range of 0.1 to 1% (w/v) coal loading [6,9]. Gokacy et al. [12] investigated the effect of increased coal loading on coal biosolubilisation. They observed that 10% (w/v) coal loading resulted in a higher release of colour compounds into the supernatant than 5% (w/v) coal loading.

Quigley et al. [13] reported on the correlation between pH and biosolubilisation of oxidised, low rank coal. They observed an increase in pH from 6.5 to 7.5 and corresponding increase in absorbance at 450 nm from 0.0 to 0.5 during solubilisation of treated coal by *Streptomyces setonii*. Holker et al. [14] also suggested that the growth of fungi on carboxylic groups from the coal macromolecule leads to an increase in culture pH. Conversely, Larboda et al. [10,15] reported that biosolubilisation of untreated coal does not influence pH. They observed that variation in pH of the supernatant did not correspond to the observed increase in absorbance.

In this study, we report on the biosolubilisation of low rank coal sourced from SASOL (SA) by the fungus *Trichoderma atroviride* in a slurry system at shake flask scale. The study quantifies the degree of solubilisation obtained under these culture conditions, as well as the effect of solids loading and particle size on microbial solubilisation performance.

2. Experimental

2.1. Coal

Sub-bituminous coal from SASOL (SA) was used. Coal samples were dry sieved into nominal size fractions of 1500–2000 μm, 600–850 μm and 150–300 μm using laboratory sieves. The dry coal samples were autoclaved at 120 °C for 20 min. No further pretreatment was performed.

2.2. Micro-organism

The fungal strain *T. atroviride* ES 11 was obtained from the laboratory of Prof. D Cowan at the University of the Western Cape, South Africa. It was maintained on agar slopes (3% agar) on the standard growth media (defined below) at 4 °C for up to 6 months.

2.3. Inoculum preparation

The pre-inoculum was prepared by culturing the fungus from the stock culture on agar plates using the growth medium. Following 5 days of culture, five plugs of fungal culture were cut from the stock plates with a sterile Pasteur pipette and inoculated into 100 ml sterile growth media containing 0.1% coal. Ten glass beads (6 mm in diameter) were added and the culture was grown for 4 days at 28 °C in an orbital incubator shaker at 120 rpm. The inoculum was prepared by transferring a 10 ml aliquot of the pre-inoculum culture into 100 ml of fresh growth medium and cultured for 2 days at 28 °C with agitation at 120 rpm.

2.4. Growth medium and culture conditions

The growth medium used for stock culture, inoculum development and experiment contained (per litre): 1 g $NH_4(SO_4)$, 3 g malt extract, 0.52 g $MgSO_4.7H_2O$, 7.6 g KH_2PO_4, 0.005 g $FeSO_4.7H_20$ and 0.003 g $ZnSO_4.7H_2O$. The standard growth medium was supplemented with 10 g glucose per litre, unless otherwise stated. Where coal was used as carbon source, the fungal culture was established by growth on glucose for 5 days, unless otherwise stated. On its depletion, coal was added to the medium and culturing continued.

2.5. Investigation of particle loading and size

The key operating variables of the slurry affecting coal biosolubilisation, namely coal loading and particle size were investigated using the factorial design detailed in Table 1. A 10 ml aliquot of the pre-inoculum culture was added into 150 ml medium in 500 ml

Table 1 – Experimental conditions for factorial experiment

Flask number	Coal loading (% w/v)	Particle size (mm)
1	5	600–850
2	10	600–850
3	5	1500–2000
4	10	1500–2000
5 Negative control (Un-Inoculated)	5	1500–2000
6 Positive control (No Coal)	–	–

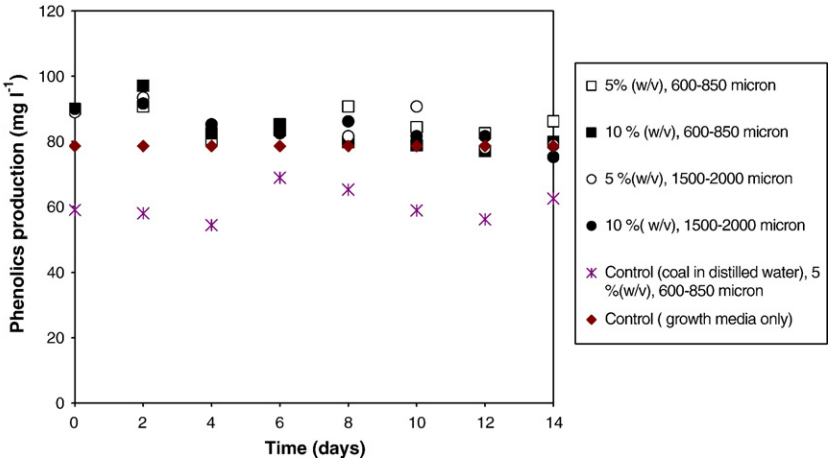

Fig. 1 – Phenolic production from *Trichoderma atroviride* ES 11 fungal strain biosolubilisation of coal in shake flask culture. Coal was added to a five-day *Trichoderma atroviride* culture on day 0.

Erlenmeyer flasks and incubated on glucose for 5 days at 28 °C with agitation at 120 rpm to establish fungal culture. Where coal was used as carbon source, coal was added to the medium on glucose depletion and culturing continued for a further 14 days. The effect of particle size fraction was investigated across the extended range from 150–300 μm to 600–850 μm under shake flask conditions using an agitation rate of 120 rpm.

2.6. *Sampling and analytical methods*

Samples of 5 ml were taken aseptically at regular time intervals (8 sample total, equivalent to 40 ml). Biomass and coal were separated from the supernatant by vacuum filtration using glass-fibre filter membranes of 0.45 μm pore size. The supernatants were analysed for phenolic content, using the Folin–Ciocalteau reagent and spectroscopic analysis at 725 nm [16]. Total organic carbon was quantified using an Anatoc Series II TOC analyser. Release of colour compounds into the supernatant was assessed by the absorbance at 450 nm. Coal weight loss was measured gravimetrically after 14 days of coal solubilisation. Following removal of the supernatant, the spent biomass was washed off with water and the residual coal samples dried at 80 °C for 48 h. Solution or suspension pH was recorded every 48 h. The microbial culture was observed microscopically at day 6.

3. Results and discussion

3.1. *Products of coal biosolubilisation and their analysis*

Coal biosolubilisation was quantified by measurement of the increase in absorbance of the supernatant at 450 nm owing to release of coloured compounds such as humic and fulvic acids. Further general release of organic materials into solution was observed by total organic carbon analysis (TOC). Potential for release of phenolic compounds was quantified by the Folin assay. Overall solubilisation was quantified by total mass loss of coal.

While there is no direct report of phenolic product release from coal biosolubilisation in the literature, the product of coal biosolubilisation is reported to be a mixture of polar organic compounds with a high degree of aromaticity (Scott et al.

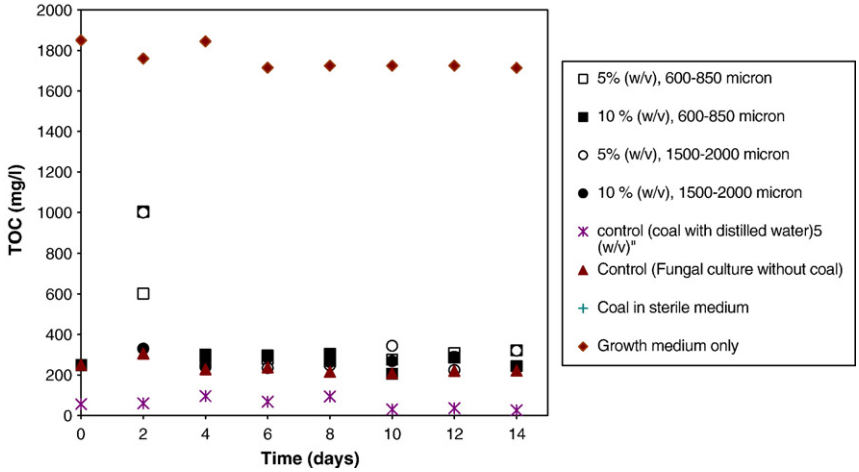

Fig. 2 – Total organic carbon concentration of fungal biosolubilisation of coal by *Trichoderma atroviride* in shake flask culture. Coal was added to a five-day *Trichoderma atroviride* culture on day 0.

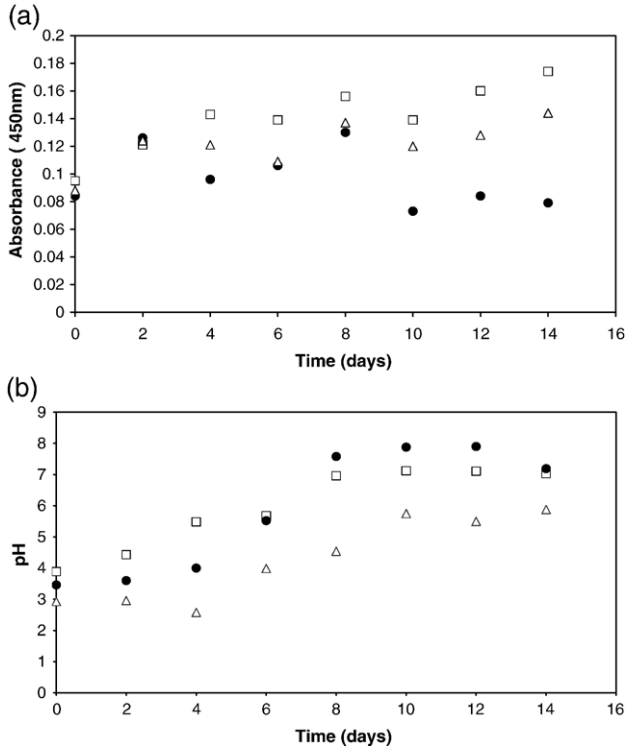

Fig. 3 – Biosolubilisation of coal in shake flask culture at a 5% loading and 600 to 850 μm diameter (□) relative to the controls of coal suspended in water (●) and the fungal culture (△), a) Absorbance at 450 nm; b) change in pH.

1986). Further, based on coal structure, release of phenolics and polyphenolics on solubilisation can be expected. Contrary to this Fig. 1 shows no release of phenolics on incubation of coal with the fungus. The phenolic concentrations in the control test containing the growth medium in the absence of coal were comparable to those measured in the presence of coal, and yielded a highest concentration of 97 mg l^{-1}. Hence the major source of phenolic compounds was the growth medium. Release of phenolic compounds on biosolubilisation of coal by *T. atroviride* ES11 was negligible.

Total organic carbon concentration (TOC) in solution provided a valuable quantification of the conversion of coal to soluble organic compounds. The change in TOC is given as a function of time during fungal coal biosolubilisation in Fig. 2. On growth of fungi on the glucose media prior to coal addition over 5 days, the TOC decreased from 1800 mg l^{-1} to 250 mg l^{-1} (data not shown). After coal addition, the TOC increased to a concentration of 330 to 1000 mg l^{-1} over a 2-day period depending on the operating conditions. A sharp decrease in TOC was observed after the second day of coal biosolubilisation, attributed to the metabolism of the soluble products released from coal to CO_2. Insignificant variation in TOC in coal slurries in the absence of fungal culture suggest that both the initial release of TOC between 0 to 2 days after coal addition and subsequent depletion of TOC resulted from microbial action rather than physical leaching.

In Fig. 3a, the change in absorbance due to the release of colour compounds is presented, comparing the biosolubilisation of coal to fungal growth and abiotic coal suspension. While increase in A450 was observed on coal solubilisation, interference from the fungal culture occurred and the changes did not correlate quantitatively with coal solubilisation. Further, the release of protons on reaction, shown as change in pH in Fig. 3b, is not dominated by the bioreaction of coal.

3.2. *Analysis of coal solubilisation*

Critical and succinct assessment of the relative merits of the techniques used to evaluate the extent of coal solubilisation is given in Table 2. Simple spectrophotometric monitoring at an absorbance of 450 nm has been widely used to determine coal biosolubilisation; however, as shown in Fig. 3a, it is qualitative. The coal weight loss method is not widely used to determine coal solubilisation because it is tedious; however results from this study showed that it gave a reliable quantitative measurement of coal solubilisation. There was no release of phenolic compounds during coal solubilisation (Fig. 1); hence they cannot be used to determine the extent of coal solubilisation. In addition, results from pH measurement, shown in Fig. 3b, suggested that there were other dominant acid-consuming reactions interfering with coal solubilisation, hence pH measurement is not recommended as a method to determine the extent of coal solubilisation. Measurement of total organic carbon (TOC) provided a qualitative method for monitoring coal solubilisation and is useful to define the soluble components in the carbon balance; however it is further metabolised as shown in Fig. 2.

3.3. *Coal biosolubilisation as a function of particle size and loading*

The effect of coal loading and particle size on coal solubilisation was investigated simultaneously through factorial analysis across the particle range 600–850 μm and 1500–2000 μm and the solids loading range 5% and 10%.

Fig. 4 shows colour measurement as absorbance at 450 nm as a function of time under the experimental conditions of the factorial experiment defined in Table 1. There was no increase in absorbance in the control tests containing growth medium only. The results also showed that there was an increase in absorbance for both fungal culture with coal and the control containing fungal culture only. The increase in absorbance was

Table 2 – Analytical methods used in coal biosolubilisation

Parameters	Qualitative/quantitative	Interferences noted	Used in literature	Recommendation
Absorbance	Qualitative	No	Widely used	Yes
Weight loss	Quantitative	No	Not widely used	Yes
Phenolics		No release	Not used	No
TOC	Qualitative	Intermediate Metabolised	Not widely used	Yes
pH	Qualitative	Other dominant acid consuming reaction	Not widely used	No

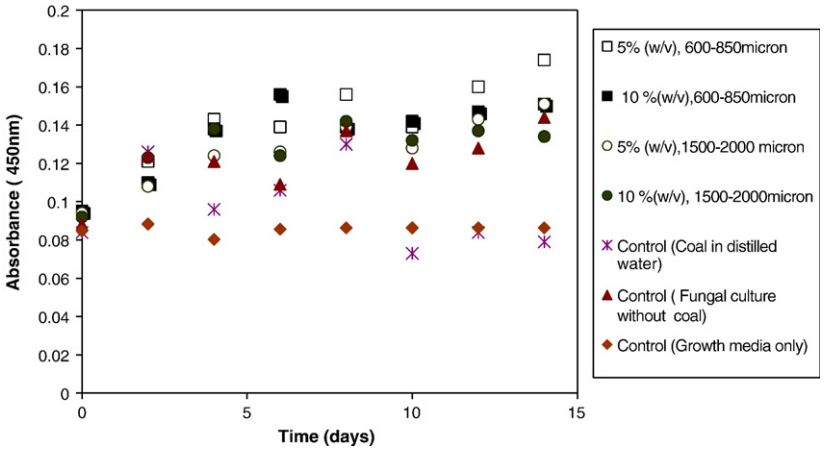

Fig. 4 – Biosolubilisation of coal by *Trichoderma atroviride* (ES 11) in shake flasks, measured in terms of colouration of the supernatant(A450). The coal was added to a five-day *Trichoderma atroviride* culture on day 0 of the experiment.

higher in the fungal culture with coal than in the fungal culture only. This suggests that there may be a correlation between release of colour and coal solubilisation. The highest increase in absorbance occurred at 5% (w/v) coal loading and 600 to 850 μm particle size fractions. This suggests that a decrease in particle size led to a higher degree of biosolubilisation.

An increase in coal loading of the same particle size fraction is expected to lead to an increase in absorbance owing to increased substrate availability. However, from our results, 5% (w/v) coal loading resulted in higher coal biosolubilisation in terms of colour increase than the 10% (w/v) case. This suggests that optimum coal loading may occur between 5 and 10% (w/v). Increase above this optimum may be postulated to lead to inhibition of micro-organisms (ES11) that are involved in coal biosolubilisation owing to cell damage as reported by Nemati and Harrison [17] and Harrison et al. [18] for bacterial and yeast systems. This was confirmed by monitoring the microbial culture under both conditions microscopically (Fig. 5) where fragmentation of the fungal mycelium was observed at 10% (w/v) loading.

Reduction in coal weight has been used as a quantitative tool to determine coal solubilisation [15]. Fig. 6 shows coal weight loss following 14-day incubation with *T. atroviride* (ES11) as a percentage coal conversion. The smaller particle size resulted in higher coal weight loss in accordance with coal biosolubilisation was a surface phenomenon. There was very little coal weight loss in the control experiments containing coal and distilled water, confirmed that degradation of coal was mediated by the fungus or associated enzymes rather than a chemical leach reaction. In the factorial experiment, the highest degree of coal biosolubilisation of 9.3% in terms of percentage dry weight loss was found at 5% (w/v) and 600–850 μm.

There was an increase in pH in the fungal culture grown in the presence of coal (change in proton concentration of 238 mM), in the control where fungi were grown in the absence of coal (change in proton concentration of 578 mM) and the control where coal is incubated in distilled water (change in proton concentration of 330 mM). The consumption of protons under each experimental condition is presented in Table 3. The change observed in the presence of both coal and fungi was independent of coal loading, and was not directly related to the extent of coal solubilisation. Hence the data presented here support the findings of Larboda et al. [10,15] in that variation in pH does not correlate with coal solubilisation on loading.

3.4. *Analysis of factorial experiment of coal solubilisation*

The use of a factorial experimental design allows a study of multi-parameter processes in which the dominant

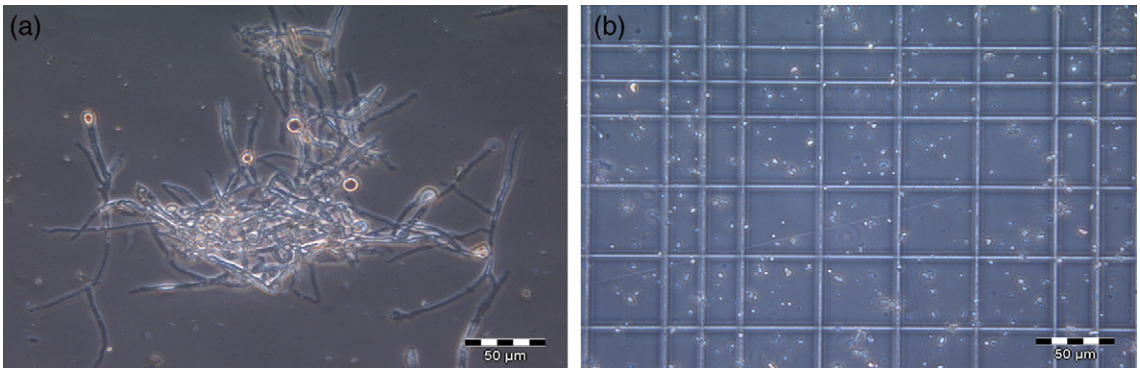

Fig. 5 – Micrographs of coal solubilisation at day 6 of *Trichoderma atroviride* growing with coal as carbon source at (A) 5%(w/v) and (B) 10%(w/v). Experiments were conducted in shake flasks. Magnification 40×.

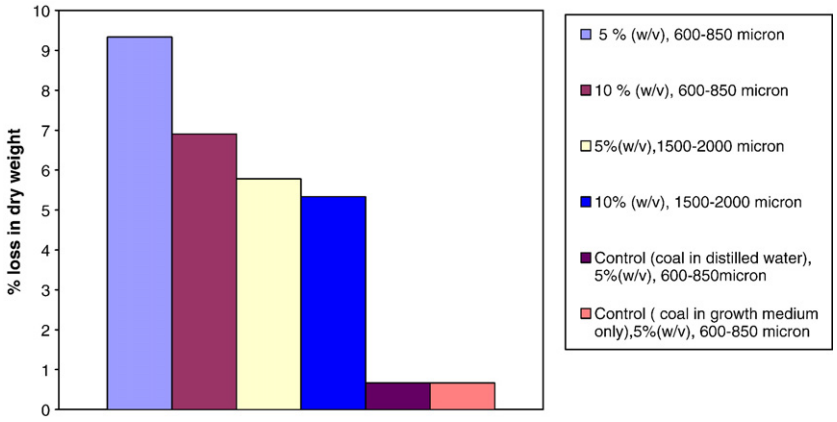

Fig. 6 – Coal biosolubilisation as a measure of decrease in percentage dry weight in relation to the initial coal weight. Factorial experiments were conducted in shake flasks. The coal was added to a five-day *Trichoderma atroviride* culture on day 0.

variables and interactions can be established. In addition, it aids the search for optimum conditions [19]. In this study, a two-level factorial design was employed to investigate the parameters of coal loading and particle size fraction. In terms of the measured response, gravimetric weight loss was used since it provided a quantitative measure of coal biosolubilisation.

Yates' procedure provides a simple method of analysing a 2^n factorial experiment to provide a simple linear model:

$$Y = \beta_o + \beta_1 x_1 + \beta_2 x_2 + \beta_{12} x_1 x_2 \quad (1)$$

where Y is the measured response, x_1 is the variable i. The significance of algorithm is that it facilitates the determination of the algebraic signs of the coefficients needed for calculating the main and interaction effects of each factor in a factorial experiment. Each factor is coded with a low level denoted −1 and the high level denoted +1.

The coal weight loss data and factorial combination are presented in Table 4. The estimate of the effect of each variable and their interaction was computed by Yates algorithm, shown in Table 5.

An empirical relationship was developed from the factorial analysis to provide a prediction of coal biosolubilisation in terms of percentage weight loss (Y) in terms of particle size fraction (b), coal loading (a):

$$Y = \frac{1}{2}\langle 17.79 + (-2.38)A + (-6.08)B + (1.83)AB\rangle \quad (2)$$

where 17.79 is the average response and A and B can be +1 or −1. Eq. (2) can be decoded to the actual values of particle size and coal loading reported in Table 4. This is shown below:

$$Y = 8.895 - 1.19\left[\frac{a - 0.5(10+5)}{0.5(10-5)}\right] - 3.04\left[\frac{b - 0.5(1.75+0.725)}{0.5(1.75-0.725)}\right] + 0.915\left[\frac{a - 0.5(10+5)}{0.5(10-5)}\right]\left[\frac{b - 0.5(1.75+0.725)}{0.5(1.75-0.725]}\right] \quad (3)$$

Further simplification gives

$$Y = 26.43 - 1.38a - 11.28b + 0.713ab \quad (4)$$

where *a* is % loading and *b* is nominal diameter (mm).

Eq. (4) suggests that the particle size fraction, coal loading and the interaction between particle size and coal loading affects the degradation of coal. The coefficient in the linear model shows that particle size fraction (b) is the most important variable and subsequently the coal loading and the interaction between both variables.

Table 3 – Analysis of the change in pH in the factorial experiment

Condition	Initial pH	Final pH	$\triangle H^+$ (M)	Coal weight loss (%)
5%(w/v), 600–850 μm	3.8	7.0	−0.0215	9.3
10%(w/v) 600–850 μm,	4.0	7.2	−0.0176	7.0
5%(w/v) 1500–2000 μm,	3.5	7.0	−0.0293	6.0
10%(w/v) 1500–2000 μm,	3.6	6.8	−0.0262	5.5
Coal only	3.4	7.8	−0.0330	0.5
Fungi only	2.8	5.8	−0.0578	

Run 1 and 2 were independent.

Table 4 – Coal weight loss in the factorial analysis replicates

Factor		Coal loading	Initial wt	% Coal weight loss (run 1)	% Coal weight loss (run 2)	% Total coal weight loss
Size	Loading					
a	b					
−1	−1	5%, 600–850 μm	7.5	9.3	18.76	28.09
+1	−1	10%,600–850 μm	15	7.0	12.77	19.67
−1	+1	5%, 1500–2000 μm	7.5	5.78	5.16	12.28
+1	+1	10%,1500–2000 μm	15	5.3	5.26	11.12

Table 5 – Effect estimate and sum of squares estimated using Yates method of statistical analysis

Combination	Total response	(1)*	(2)#	Effect estimate	Sum of squares (SS)
(1)	28.09	47.76	71.23	Total	188
A	19.67	23.46	−9.53	−2.38	22.7
B	12.28	−8.43	−24.30	−6.08	148
Ab	11.12	−1.10	7.32	1.83	10.4

1)* — The first entry in column (1)* represents the sum of the response of the first two entries in response column (i.e (1) + a). The second entry is the sum of the second two entries in the response column (i.e b and ab). The third entry is the difference between the first two entry responses. (i.e a-(1)).The fourth entry is the difference between the second pair of responses (ab-b).
(2)# — The same procedure is applied using data in column (1)*.

3.5. *Extending the operating window of particle size*

Owing to the dominant effect of size, it was desirable to extend the operating window of particulate size considered. Experiments were carried out at 600–850 μm and 150–300 μm at 5% (w/v) coal loading. The effect of particle size on coal solubilisation is presented in Fig. 7. Results from both qualitative (release of coloured compounds measured as absorbance at 450 nm) and quantitative (decrease in dry mass of coal) analyses of coal biosolubilisation show that extent of solubilisation of the 150–300 μm particle size fraction and the resultant accumulation of coloured products in solution from this fraction was increased. The extent of coal degradation was increased by 4-fold when using a size fraction of 150–300 μm in comparison with the 600–850 μm fraction. Coal biosolubilisation is a surface phenomenon, demonstrated by obtaining better biosolubilisation when a smaller size fraction is used (Gokacy et al. 2001). In Table 6, coal solubilisation is presented as a function of nominal particle surface area (assuming spherical particles). The extent of decrease aT150–300 μm with a specific surface area of 2.17 $cm^2\ g^{-1}$ was 28% coal weight loss compared with a

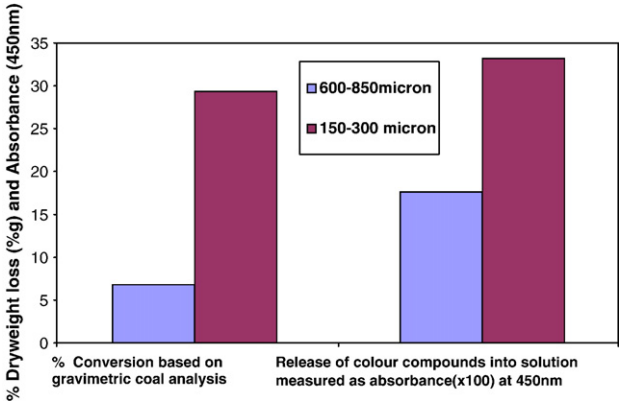

Fig. 7 – Effect of particle size fraction on coal solubilisation during coal biosolubilisation with *Trichoderma atroviride* ES11.

Table 6 – Effect of particle size fraction and available surface area on coal biosolubilisation

Particle size fraction	Initial coal	Mass solubilised	Specific surface area ($cm^2\ g^{-1}$)	% Coal weight loss
150–300 μm	7.5	2.5	2.17 $cm^2\ g^{-1}$	28
600–850 μm	7.5	0.6	0.54 $cm^2\ g^{-1}$	7.8
1500–2000 μm	7.5	0.375	0.21 $cm^2\ g^{-1}$	5.6

7.8% coal weight loss at 600–850 μm (0.54 $cm^2\ g^{-1}$) and a 5.6% coal weight loss at 1500–2000 μm (0.21 $cm^2\ g^{-1}$).

4. Conclusion

This study has shown that coal weight loss and colour absorbance can be used to monitor coal biosolubilisation. Particle size fraction, coal loading and the interaction between the particle size and loading affect the coal biosolubilisation process. Under the conditions studied in the factorial experiment, the highest degree of coal biosolubilisation was at 5% (w/v) coal loading using particle size fractions of 600–850 μm. The fungal growth was inhibited at 10% (w/v) coal loading. Coal biosolubilisation was enhanced by increased availability of surface area achieved either by increased loading (up to the critical value) or reduced particle size. Decreasing the particle size fraction from 600–850 μm to 150–300 μm resulted in a 4-fold increase in surface area with an associated increase in the degree of coal solubilisation of 4-fold. There was a 28% coal solubilisation of particle size fraction of 150–300 μm at 5% coal loading.

No significant increase in phenolics concentration was observed on coal biosolubilisation, Hence phenolic compounds are not a product of coal biosolubilisation. Further, the TOC analysis suggested that, following release of soluble organic compounds from coal, these are further metabolised to CO_2 since no significant increase in TOC was maintained through the experiment.

Acknowledgement

We would like to acknowledge the financial support of the National Research Foundation of South Africa and the University of Cape Town for the present work.

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