

Safety in Mines Research Advisory Committee

Phase 2 Project Report

Inertisation of sealed coal mine panels using microbial organisms

D P Creedy, C Burton, A Sharman, H R Phillips and J J Alexander

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Executive Summary

This is the second part of a two part study examining the possibility of using naturally occurring methane-oxidising organisms to prevent the accumulation of flammable mixtures in sealed areas within coal mines. The first phase reviewed previous work undertaken in various coal mining countries which demonstrated the potential of the technology subject to overcoming some problems relating to scale.

A biological control solution would appear to be suited to the combination of relatively low methane emissions and favourable climatic conditions in South African bord-and-pillar workings. Various methods could be used for applying microbial systems involving spraying roadway surfaces, suspending activated carbon bio-curtains in roadways or circulating the mine atmosphere through a biofilter. The logistics and cost will depend on whether treatment is applied to whole abandoned areas or selected areas in which gas hazards and risks have been identified.

Current reasoning favours the bio-curtain approach applied to selected areas together with an irrigation facility through which water, nutrients and fresh culture can be sprayed as required.

The next step is to conduct a field trial to prove the technology under mining conditions and facilitate the development and costing of a production system.

1 Introduction

1.1 Control of risk from abandoned areas

Worked-out bord and pillar sections in South African coal mines are usually sealed on abandonment to increase the availability of air for new working faces where high standards of ventilation are essential for both gas and dust dilution. Methane concentrations in the ventilation air are usually low and often undetectable due to dilution (low initial gas levels in large airflows) but when ventilation is stopped significant concentrations of methane can accumulate over a period of time. In the vicinity of imperfect seals potentially flammable methane-air mixtures can be formed.

The quality of sealing depends on ground conditions and the method of constructing stoppings, the latter being constrained by time and cost. A widespread requirement for explosion resistant stoppings could be too costly to sustain. Consequently, biotechnological methods are being examined as a possible means of controlling the risks and hence improving the safety of workers in coal mines.

1.2 Biotechnology

Biotechnology is being increasingly used in the mining industry and there are many examples of bioprocesses and biotreatments used commercially in mines throughout the world. However, most of the scientific work on methane has focused on methane production from raw wastes (for energy purposes) rather than methane removal. However, there have been some laboratory experiments and fields trials using methane-oxidising microbes to lower methane concentrations:



Significant quantities of methane can be oxidised by these methanotrophs. Methane oxidation rates are dependent on various factors including the type of methane oxidising microbe, pH, moisture, oxygen availability and methane availability. Laboratory studies have demonstrated methane oxidation rates by pure cultures of methanotrophs from 10 to 31 mmol CH₄/g/h. In comparison, methane oxidation rates measured in natural samples such as soils, peat and sediments range between 0.002 and 10 mmol CH₄/g/h. Specific methane emission rates vary according to type of sample (wetland sediment, sediment, peat, lake soils) and generally vary from 0.1 to 100 mg CH₄/m³/d.

Conditions in South African collieries are favourable for the growth and propagation of methane oxidising microbes and their use in controlling methane in sealed areas. Temperatures lie typically in the range 15 – 24°C, humidities from 80 – 95 per cent and methane emission rates are relatively low.

Practical application and demonstration of this biotechnology will require an interdisciplinary approach to facilitate the development of a robust and efficient technology suitable for use in coal mines.

1.3 Inertisation

This is the second part of a two part study examining the possibility of using naturally occurring methane oxidising organisms to prevent the accumulation of flammable mixtures in sealed areas. The methane oxidising (methanotrophic) organisms convert the methane into carbon dioxide thus producing a largely “inert” atmosphere. In the absence of oxygen, biological activity will cease. This does not limit the effectiveness of the process as there is no ignition or explosion risk without

oxygen present and methanotrophs can survive temporary absence of oxygen without permanent detriment.

Strictly, the process generates a non-flammable rather than an absolutely inert atmosphere. In principle, the degree of inertisation achievable is likely to be comparable to that obtainable by nitrogen injection where an oxygen concentration of 10 per cent or less is usually regarded as sufficient to prevent methane explosions and 6 per cent to protect against hydrogen mixtures (Nicolay, Mark and Mallett, 1999)

1.4 Objectives of Phase Two

The first phase included a review of relevant literature and descriptions of previous work and confirmed the availability of the technology. This second phase is concerned with validating the technology in South African mining conditions and outlining the requirements for an underground pilot trial.

The objectives of Phase Two were to:

- Obtain minewater samples from South African collieries.
- Isolate and grow suitable organisms in the laboratory.
- Design a practical method for implementing the biotechnological solution.
- Clarify operational, safety and health risks associated with the proposed application.
- Specify the requirements of a pilot trial.
- Prepare a Phase Two report and an industry briefing note.

2. Laboratory studies in South Africa

Laboratory work was undertaken in the Department of Molecular and Cell Biology (Microbiology) at the University of the Witwatersrand under the direction of Professor J J Alexander.

Methane-oxidising micro-organisms were isolated from two South African minewater samples and cultures were grown (Appendix 1) in air containing 2.5% methane by volume. The presence of methane-oxidising micro-organisms was established but the growth rate was slow. More active organisms were isolated from stagnant pond water.

Importantly, the laboratory testing demonstrated that methane-oxidising microbes could “hibernate” in the absence of methane. It is therefore likely that the organisms could survive the lack of methane during sealing-off, and become active if a build-up of methane occurred.

3. Biotechnological options for methane removal

A microbiological system is required which can be installed prior to sealing off a worked-out area of a mine and which will remain active for a minimum of, say, 20 years. Although methane-oxidising micro-organisms are reasonably robust and tolerant of a range of ambient conditions, they may not survive, or remain consistently active, unless certain essential ingredients are available. Nutrients and trace elements may need to be replenished at intervals, otherwise serious die-back could occur in the temporary absence of methane or oxygen. The availability of moisture is particularly important. An installation may therefore also require provisions for maintaining the biological system in a suitably active state. A “one shot” install and forget approach would be preferable but may not be a practical option at all locations.

The issues that need addressing are:

- (a) Techniques for applying the microbes,
- (b) Sustainability of the biological process,
- (c) Treatment configurations to provide a cost effective solution for mines, and,
- (d) Remote gas monitoring to confirm the adequacy of the system.

3.1 Techniques for applying the microbes

Three possible application methods, which have been demonstrated in principle elsewhere, could be considered for use in South African mines:

- Pump and spray technology to coat roadway surfaces.
- Use of methane oxidation units or biofilters through which the mine atmosphere is circulated.
- Suspended support media in the form of curtains or drapes attached to the roof.

The merits and disadvantages of each of these methods are listed in Tables One, Two & Three.

3.1.1 Coated roadway surfaces

Microbial cultures will not necessarily freely adhere to roadway surfaces. Fixing agents (polymers) will be needed to help attachment and to assist microbe immobilisation onto coal roadway sides. However, the fixative is likely to reduce contact between gas and microbes.

Table 1 Pump and spray technology

Method: Methane-oxidising microbes applied directly to roadway surfaces by spraying	
Where used: Former Soviet Union	
Advantages	Disadvantages
Simple engineering Large area easily treated Low maintenance requirements Relatively low cost	Low density coverage Poor contact between microbes and gases Possible problems with desiccation Low efficiency

Table 2 Methane-oxidising units or biofilters

Method: Gas actively drawn through a device containing methane-oxidising microbes	
Where used: Laboratory scale experiments in the UK, Australia and USA	
Advantages	Disadvantages
Good contact between gases and microbes High methane removal efficiency Whole sealed areas could theoretically be treated Unit accessible for maintenance Controlled reactor conditions	Engineering complexity External power requirement Maintenance requirements Low treatment flow rate Higher cost than spray option

Table 3 Passive bio-curtain filters

Method: Passive removal of gases by microbes immobilised on suspended curtains	
Where used: Former Soviet Union	
Advantages	Disadvantages
Passive system Good contact between gases and microbes High methane removal efficiency	Maintenance requirements Higher cost than surface spraying option May restrict gas movement

Whole sealed areas could theoretically be treated	
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3.1.2 Bio-filters

A biofilter is a type of methane scrubber. Biofilter designs have been based on the use of liquid cultures in fermentor type reactors or fixed systems where microbes have been immobilised on to an inert support within a mechanical unit. These systems have only been tested under laboratory conditions but with positive results.

A system for mine use would incorporate a reactor unit, condition monitoring, pipework, valves and an approved gas pump driven by a suitably protected electric motor. Heat would be emitted by the unit and some elementary cooling may be necessary to maintain the cultures at an optimum temperature. The unit could be sited in fresh air with pipework leading through stoppings for extracting methane rich atmospheres from the sealed areas and discharging methane depleted, carbon dioxide rich gases into them. Extraction and discharge locations would be widely separated to minimise short-circuiting of flows. The physical size of the unit would depend on the flow rate to be treated. A dry-seal pump would be preferred, subject to regulations. A water-seal pump could be used if necessary but the cost would be substantially higher (up to an order of magnitude) and the efficiency lower than a dry-seal extractor. A recirculation valve may be needed to enable low flows to be achieved.

3.1.3 Passive bio-curtain filters

This option envisages the use of specially treated mats or curtains suspended in the sealed off roadways. A passive system of this type would not require mechanical assistance as needed with the biofilter although, in some circumstances, the curtains could hinder gas movement in their vicinity so reducing the effectiveness of the system. Nevertheless, concentration gradients and thermal gradients would probably induce sufficient flow to remove products of reactions and introduce fresh methane to the microbes.

Curtains offer distinct advantages compared with roadway surface treatments, in that, with the appropriate choice of materials, they can:

- Adsorb methane from mine air bringing gas into intimate contact with the immobilised microbes
- Provide a surface and support medium for methane-oxidising microbes to grow on.

Methane-oxidising bacteria are tolerant to low moisture and are able to recover following drying. For this approach, carefully selected methane oxidisers would be immobilised at high concentration onto the mats. The mats would then be suspended from the roadways and activated by spraying with water and nutrients.

Several support materials have been considered including PVC/polypropylene, polyester woven fabric, foam, activated charcoal cloth (ACC) and even coal. Carbon based materials are preferred due to the benefits of methane adsorption.

This option offers several advantages, in particular simplicity of use (especially to operators who have little or no knowledge of microbial processes) and potentially high efficiency. At this stage, it would appear to represent the most practical method for application in coal mines.

The key features of a successfully engineered application would include:

- Immobilisation of selected methane-oxidising bacteria on a support cloth matrix (e.g. ACC).

- Fixing and positional alignment of bio-barriers within the sealed coal mine panels to ensure some air movement along and through porous material.
- Installation of a spray device for intermittent (probably only a few times per year) supply of water, nutrients and, if required, further inoculum.
- A facility to remotely monitor gas concentrations in the sealed area.

3.2 Process sustainability

Neither the surface spraying or the suspended curtain application methods can be guaranteed to provide the required protection over long periods of time without some attention. Permanent spray systems would be needed to deliver fresh culture, nutrients and water as required to ensure longevity. These arrangements would need to be installed prior to sealing of the abandoned area. Once sealed off, no maintenance would be possible. The replenishment system would therefore require careful engineering and installation.

3.3 Treatment configuration

There are two possible approaches to the implementation of the bio-technology in terms of the area to be treated. The treatment can be applied to either the whole of an abandoned area or just to selected parts where the most significant gas hazards are considered to originate.

3.3.1 Whole area treatment

The passive methods involving surface coating or suspended curtains could be applied throughout an area at abandonment. However, it would be impracticable to install an irrigation system capable of spraying all the support material due to the complexity and cost. Provided humidity was high, which is likely in a sealed area, a high density of treatment may adequately compensate for any reduction in reaction rate over time. A full-scale trial would be needed to investigate the effectiveness of this approach and also to provide design data to facilitate optimisation of future installations.

Conditioning of a void volume of up to say 500,000m³ would require a large biofilter unit to inertise the atmosphere to a safe condition within a reasonable period of time. At a process rate of 1m³/min, almost one year would be required to treat the air volume once. This would not appear to represent a practical proposition.

3.3.2 Selected area treatment

Rather than attempt to treat the whole of a sealed area, which may be prohibitively costly, treatment could be concentrated in a specific area. The identified area may be disturbed ground or a faulted region in which significant gas emissions were detected during development.

Alternatively, the treated area could comprise a selected number of roadways immediately inbye of the stoppings. Thus, an “inert” barrier would be created between the working mine and the sealed area. Inertisation could be achieved using surface coating or curtain methods, or alternatively, a modestly sized external biofilter unit.

This approach would be more cost-effective than whole area treatment provided that the “biological barriers” were appropriately located. The aim would be to design an installation to address site specific gas hazards and risks. With a limited area of treatment, irrigation sprays become a practicable proposition to ensure the longevity of suspended curtain systems.

3.4 Remote gas monitoring

Some form of gas monitoring would be advisable to confirm the efficacy of any installed biotechnological system or to provide an indication of when remedial spraying is required, if available. A tube bundle sampling arrangement, installed prior to abandonment, could be used to draw gas mixtures to an analyser situated either on the surface or at a convenient underground location. Further details and options may be found in the results of the contemporaneous SIMRAC project COL 602 "Monitoring of atmospheres in inaccessible areas of coal mines".

4. A practical technology for South African coal mines

4.1 The preferred approach.

Having examined various methods for introducing methane bearing gases to oxidising microbes, careful consideration is warranted of the likely cost effectiveness and mining engineering constraints on implementation of the options. On balance, the initially recommended approach would be to pursue the suspended curtain method. The reasons for this are:

- Ease of transport and installation
- Lower development and manufacturing cost than a biofilter unit
- No power requirement
- Potential high efficiency of methane removal
- Simple maintenance
- Availability of suitable support materials.

4.2 Selection of micro-organisms for immobilisation

Several methane-oxidising microbes have been widely used in methane control research and mining projects, including:

- *Methylococcus thermophilus* (USSR)
- *Methylomonas rubra* (USSR)
- *Methylominus trichosporium* (US, UK, USSR)
- *Methylococcus capsulatus*. (USSR)
- *Methylomonas methanica* (US)
- *Methylomonas fodinarium* (Australia)

Samples of mine water have been obtained from some collieries in South Africa and methane-oxidising microbes isolated for comparison with cultures described in other countries. Following initial confirmatory laboratory tests, it is recommended that comparisons are made of relative methane oxidation rates of South African cultures with methane-oxidising microbes from national culture collections.

Methane-oxidising microbes such as *M. trichosporium* and *M. capsulatus* can be grown in fermentors in order to achieve high biomass production. The fermentation approach will allow grams per litre production of cells, which then are used for immobilisation on the support matrix. In general, the optimum temperature and pH for high methane monooxygenase activity is 30-35°C and pH 6.2-6.4 respectively. High biomass productivity of over 0.5g/l/h is achievable under these controlled conditions. A high cell count should be achieved, in excess of $10^6 - 10^8$ cells/ml, which will provide a suitable inoculum for fixing on to the mats.

Typically, the active cell numbers will drop initially but as the microbes acclimatise to conditions on the support and the environment, there will be an increase in methane-oxidising activity due to increase in cell numbers and biofilm formation.

4.3 Development of the bio-curtain technology

Microbes immobilised onto a fixed support matrix are capable of achieving higher rates of activity than when available in suspended culture. The intention of the biotechnological curtain approach is to immobilise a consortium of highly active methane-oxidising microbes onto a suspended support matrix or filter.

A suitable support material will have a number of properties, which include:

- high external surface and relatively porous to allow free transfer of air/methane
- non-inhibitory to bacteria
- high adsorptive capacity of methane
- durable
- wetting capability

Activated charcoal cloth (ACC) is recommended as a fixed support for these microbes. ACC is 100 per cent activated charcoal produced as a textile by Charcoal Cloth International (a subsidiary of Calgon Corporation USA), Tyne and Wear, UK. A range of sizes and porosity is available but a 220 g.m² product with an internal surface area of approximately 1200m² g⁻¹ would appear to be suitable. The choice of ACC has been made on the basis of the following advantages:

- May be available on-site and used by mine operators for other purposes.
- High adsorptive capacity (Figure 1) for volatile organic molecules due to the presence of activated charcoal.
- Versatile for operation as a suspended filter due to ease of handling, flexing properties and does not suffer from ageing.
- Adsorption capacity less affected by pre-adsorbed moisture than granular activated carbon and therefore more suitable for use in humid environments.

Microbes will be able to colonise the porous matrix (Figure 2) and form an active biofilm on the cloth. It will be crucial to ensure that the suspended cloth is kept sufficiently moist and supplied with nutrients to allow the microbes to grow and oxidise methane efficiently. Microbes not only require available nitrogen and phosphorous (C:N:P ratios 10:5:1) nutrients for growth but will also require a low concentration of trace elements essential for good biofilm formation. This trace element solution may contain a very low concentration of copper, as this is an essential component for methane monooxygenase activity (the key enzyme for methane oxidation).

The initial immobilisation of the microbes can be achieved by gradual drying of cloth immersed in a concentrated suspension of microbes and nutrient. Following installation, and during operation in the sealed area, the cloth may require further inoculation of active cultures. This could be achieved using a fixed spraying system (Figure 3) as microbes can withstand high shear stresses.

4.4 Positioning of bio-curtains and spray nozzles

The microbially active bio-curtains would be positioned to encourage the air/methane flow to pass across and through the porous structure. The bio-curtains could be suspended or draped to maximise their exposure to the mine atmosphere. Pleating could be examined as a method for further increasing the surface area to volume ratio and hence the efficiency of the curtains.

An array of sprays positioned above and along the bio-curtains would provide a means of supplying water, nutrients and microbial culture, as required. However, it should not be overlooked that regular spraying of moisture and nutrients may also encourage growth of biofilm on the roadway sides which will in themselves become sites of methane-oxidising activity.

4.5 Design and installation of an irrigation system

The use of spray applications in industry is widespread and the technology is well proven. It is crucial that any spray systems to be installed in a sealed area have been demonstrated as reliable in mining or similar environments.

The essential operating data of spray nozzles are flow rate, spray angles, liquid distribution (solid or hollow cone), spray impact and droplet size. All of these parameters should be optimised prior to installation. Some thought will be needed when selecting an appropriate nozzle type which must not be prone to clogging.

A suitable pipe diameter for spraying can be estimated from engineering information and a knowledge of the moisture flow required for the bio-curtains. An environment in which the cloth maintains a moisture capacity of between 15 – 25 per cent should be achieved.

4.6 Installation of remote monitoring equipment

A means of monitoring methane concentrations at various locations within the sealed area is desirable and a suitable method has been described above in 3.4.

4.7 Factors affecting bio-curtain performance

Several key factors will affect the efficiency of bio-curtain technology:

- Type of methane-oxidising microbes – locally isolated cultures are likely to be just as effective as ones from cultures banks, although laboratory tests are needed to validate methane oxidation rates.
- Concentration of methane-oxidising microbes on the support – up to 1kg/m^3 depending on the type of support and microbe.
- Rate of methane oxidation - typical microbial CH_4 oxidation rates vary from 0.7 to 237 mmol/g dry wt./h.
- Oxygen within the sealed off area – a fundamental requirement for microbial oxidation of methane
- Air movement - will assist gas/microbe contact.
- Nitrogen, phosphorus and trace elements – required supporting microbial metabolism and methane oxidation. They will be present in mine water, but a nutrient supplement should be sprayed on a regular basis.
- Temperature – most methane-oxidising microbes isolated and tested tend to grow best at 25 to 35°C although microbes capable of growth at temperatures as high as 50°C . If temperatures exceed 50°C , it is likely that the methane-oxidising microbes introduced into the mine will be adversely affected.
- pH – best methane-oxidising performance will be achieved around neutral (pH 6-8).
- Water – essential requirement for methane-oxidising microbes and high moisture levels will assist gas transportation and therefore performance. The environment is likely to have a high humidity following regular spraying.

4.8 Equipment requirements

A list of the necessary equipment for the bio-process is shown in Table 4. The surface equipment could be located at the mine site or in a central laboratory.

Table 4 Equipment requirements

Surface	Underground
<ul style="list-style-type: none"> • Air agitated fermentors – 1,500 litres x 5 These could be placed in series near to the mine and used for producing methane-oxidising microbes. Instead of using methane as a carbon source for growth, methanol could be used. Recovery is aqueous only and by batch production. • Basic instrumentation (temperature, pH, DO) plus automatic addition/feeding capabilities for nutrients. • Activated charcoal cloth – cut to size for mine area • Dip tanks (1000 l) for contacting charcoal cloth with bio-culture. • Transfer pumps and pipes • Air compressor for fermentors • Tank (20 t) for water, nutrients, microbes that will be pumped into closed mine area 	<ul style="list-style-type: none"> • Spray Nozzles and connecting pipes. • Frames containing activated charcoal cloth with bio-culture. It is envisaged that several frames would be hung on bolts or straps across the full width of roadways. • Methane monitoring equipment.

4.9 Process costs

It is difficult to estimate actual costs at this stage but cost categories can be identified and these are shown in Table Five.

Table 5 Cost categories

Fixed capital costs	Operating costs
<ul style="list-style-type: none"> • Direct costs - Processing equipment, installation, piping, electrical instrumentation • Indirect costs – bioengineering design, piping and instrumentation drawings, procurement, administration, and travel. • Start-up costs – equipment modifications to bring existing plant up to specified capacity, local personnel training, technical support, and general operating expenses. 	<ul style="list-style-type: none"> • Raw materials and supplies (manufactory and laboratory) • Labour and supervision (operation and maintenance) • Utilities (electricity and water)

4.10 Process design estimates

Basic calculations (Appendix 2) involving assumptions based on the results of previous work (see Phase 1 report) indicate the feasibility of the envisaged application. Some 11 bio-curtains, each of 9m² cross-sectional area, should be capable of treating a methane emission rate of 5 litres/min per 300m³ of void volume.

5. Recommendations for a pilot trial

5.1 Outline methodology

An approximately twelve month pilot trial should be sufficient to evaluate the technology in the underground environment.

A suggested site would consist of two, recently driven adjacent blind coal headings of similar length that could be temporarily, but securely sealed for the duration of the experiment. Bio-curtains would be installed in one of the headings. The other would act as a control. Gas concentrations in each heading would be remotely monitored using a simple tube bundle system. The proposed activities and durations are listed in Table 4.

5.2 Health and safety aspects

Methane-oxidising microbes are considered harmless and non pathogenic. They occur naturally in mines and mine workers are already likely to be regularly exposed to them. Nevertheless, the preparation of cultures and immobilised microbes should be conducted in accordance with established procedures for safe handling of biological substances. Microbes imported into the mine will be in an immobilised state and need not be irrigated and activated until in place.

On completion of the trial, all the biological materials would be removed from the mine and disposed of to an appropriately licensed facility after taking samples for laboratory testing.

Prior to introducing the bio-curtain system to the trial site, staff and workforce should be briefed to dispel any health or safety concerns. An industry guidance note is provided in Appendix 3.

Table 6 Elements of a proposed pilot trial

Activity	Duration
Site selection and safety consultations	2 weeks
Specification of site requirements	1 week
Design of irrigation and monitoring systems	2 weeks
Design of biological systems	2 weeks
Ordering and delivery of equipment	6 weeks
Site and stopping preparation	3 weeks
Inoculum and consortia preparation	4 weeks
ACC immobilised matrix (bio-curtain) preparation	4 weeks
Installation of pleated bio-curtains	1 week
Installation of spray system	1 week
Testing of irrigation and monitoring system	1 week
Activation of bio-curtains	1 day
Sealing of test area	1 day
Monitoring of methane concentrations	28 weeks
Decommissioning of site and removal of materials to surface	4 weeks
Laboratory examination of sampled biomass	8 weeks
Quarterly progress reports	1 – 2 days
Final report	2 weeks

6 Conclusion

Technical information and data have been gathered which indicate the feasibility of applying biological methods to reduce methane risks in sealed areas of South African coal mines. A field trial is now required to demonstrate the practicality of the approach and provide the basis for designing an operational system.

References

Nicolay P, Mark R, Mallett C (1999). Mine gas inertisation. CSIRO Exploration and Mining, NSW, Australia p196, 1999.

Appendix 1

Laboratory studies in South Africa

ISOLATION OF METHANOTROPHS FROM WATER SAMPLES

1. Introduction

The initial proposal indicated that known, well characterised methanotrophs would be included in the tests to isolate methane-oxidising bacterial strains from South Africa. Most well characterised strains available from the America Type Culture Collection (ATCC) have the following characteristics:-

- growth in methane concentrations of about 50%, and/or
- growth at 45C or higher.

In consultations with the Council for Scientific and Industrial Research (CSIR) in South Africa, the following facts were established:-

- the ambient temperatures in sealed coal mines would range from 20 deg C to 30 deg C
- the maximum permissible concentration of methane allowed in gas cylinders was 80% of the minimum explosive concentration.

CSIR itself had equipment that would allow for higher (explosive range) methane concentrations to be used. However, they did not have laboratory facilities, in the same location, for microbial isolation. It was therefore decided to use gas cylinders containing 2.5% methane in air. This was an appropriate concentration, representative of the dilution that would occur at the time of sealing a mine section. It was also the maximum methane concentration that the supplier would provide.

Two water samples were provided from Mpumalanga sealed coal mines, and a further sample from a stagnant pool in Gauteng.

2. Protocol for the enrichment of methanotrophs from water samples.

2.1 Materials

2.1.1 Nitrate mineral solution (per litre)

Magnesium sulphate heptahydrate 1g
Calcium chloride dihydrate 0.3g
Potassium nitrate 1g
Ferric-EDTA complex or ferrous sulphate 4mg

2.1.2 Phosphate buffer stock solution, pH 6.8, 2.9M (per 100 ml)

Potassium dihydrogen phosphate 5g
di-Sodium hydrogen phosphate dihydrate 45g

2.1.3 Trace element solution (per litre)

Boric acid 0.28g
Nickel chloride dihydrate 0.08g
Cupric sulphate pentahydrate 8mg
Manganese chloride tetrahydrate 8mg
Zinc sulphate dihydrate 0.15g
Sodium molybdate dihydrate 0.05g

3. Method

3.1 One ml of phosphate buffer stock solution and 1ml trace element solution was added to 500ml of nitrate mineral solution to yield a final phosphate concentration of 5.8mM and a final pH of 6.8 to 6.9.

3.2 One ml of water sample to be tested for isolation and enrichment of methanotrophs was then added to 25ml of supplemented, filter sterilised nitrate mineral solution and placed in a sealed 250ml glass conical flask containing 2.5% methane in air.

3.3 The flasks were incubated in the dark at 30C on an orbital shaker (93rpm) until growth was observed.

4. Results

After 2 weeks incubation, growth was obtained in nitrate mineral solution using ferrous sulphate or ferric-EDTA as the iron source.

4.1 Mine water samples 1 and 2

Very faint pellicular-type growth occurred along the periphery of the glass flasks at the liquid/gas interface. Very slow growth was noted and could not easily be subcultured.

4.2 Stagnant pond water

Much more active growth was observed, the liquid becoming turbid within 2 weeks, and the micro-organisms were readily subcultured.

4.2.1 Nitrate solution with ferrous sulphate.

Predominantly gram negative curved rods were isolated.

4.2.2 Nitrate solution with ferric-EDTA

Predominantly clumps of gram negative cocci associated with chains of gram positive cocci were isolated. These bacteria had a more rapid growth rate than those in the ferrous sulphate supplemented medium and old cultures indicated the development of cysts.

5. Discussion

The ferrous sulphate supplemented medium indicated that the methanotrophs isolated were characteristic of Type 11 methanotrophs, while the mixed cultures (both gram positive and gram negative cocci) isolated from the ferric-EDTA supplemented medium were more characteristic of the Type X methanotrophs. None of the bacteria isolated were pigmented (a characteristic of many methanotrophs). A recent search of the literature has shown that 2 new species of methanotrophs have recently been described, one isolated from soil and the other from peat (Bull ID, et al. Nature 2000, 405, pp 175-178; and Dedysh SN, et al. Int J Syst. Evol. Microbiol. 2000, May 50,pt 3: 955-969). The methanotrophs isolated in this study suggest that the ferric-EDTA supplemented isolation media give rise to fairly rapid growth of methane-oxidising bacteria at methane concentrations of 2.5% in air; and that old cultures (depleted of methane as a carbon source) appear to result in the formation of resting cysts. These bacteria could form the basis of in situ experimentation in worked out, sealed coal mine areas.

Appendix 2

Process design estimates

Initial assumptions:

A unit void volume of 300 m³ with a methane emission rate = 5 litres /min

Calculations:

1 mol CH₄ occupies 24.1 litres at 25°C

Therefore: 5 litres CH₄ x 60 (min) = 300 litres CH₄/h

CH₄ conc. = 300/24.1 = 12.4 mols CH₄

1. Typical microbial CH₄ oxidation rate (V_{max} – see Phase 1 report):

Assume 26 mmol/g dry wt./h (or 0.026 mol/g dry wt./h)

2. Quantity of methane-oxidising microbes required:

= 12.4/0.026 = 477 g dry wt (bio-culture)

3. Quantity of methane-oxidising bacteria (wet wt.)

Assume bio-culture is produced at a rate of 50 mg/litre or 50 g/1000 litre

Amount of bio-culture suspension required = 477/50 = 9.54 t

4. ACC barrier size

Assume external surface area = 9m² x 3 mm thickness

Volume = 0.027 m³

5. Microbial loading capacity

Assume : 5 g active cells/m²

6. No. of bio-curtains

If 477 g active cells required to oxidise 300 litres CH₄/h or 12.4 mols CH₄/h

Then, area of bio curtain required = 477/5 = 95.4 m²

No. of bio-barriers required = 95.4/9 = 10.6

Key assumptions made in these calculations are microbial methane oxidation rate (26 mmol/g dry wt./h) and microbial loading capacity (5 g active cells/m²) based on previous studies described in the scientific literature (see Phase 1 report).

Appendix 3

Industry briefing note

The safe use of microbial organisms for reducing methane hazards in bord-and-pillar mines

Preface

This note is aimed at mine personnel likely to be involved in the testing or application of a biotechnological system for reducing methane hazards in coal mines. The research background is briefly described and safety precautions are highlighted. The need for more detailed guidance should be assessed after a pilot trial has been completed.

Introduction

The methods of bord-and-pillar mining currently practised in South Africa usually require the sealing of a substantial number of roadways to isolate a worked-out panel from the operational mine. Methane can accumulate in these sealed areas of a coal mine and, under certain circumstances, could be ignited.

Studies have been undertaken on behalf of SIMRAC (COL 604: Inertisation of sealed coal mine panels using microbial organisms) to examine a possible risk control solution involving the use of naturally occurring, harmless micro-organisms to remove methane from underground coal mine atmospheres.

The study was led by Wardell Armstrong (an international mining research and consultancy firm) in collaboration with IBS Viridian Ltd (an international biotechnology company) and the University of the Witwatersrand (Mining and Biological Sciences Departments). Laboratory work was undertaken in the Department of Molecular and Cell Biology (Microbiology) at the University of the Witwatersrand under the direction of Professor J J Alexander.

The report concluded that:

- Research in Australia, Canada, the former Soviet Union, India, the UK and the USA demonstrates that methane-oxidising micro-organisms could remove methane from coal mines.
- A bio-technological system could control the relatively low methane emissions in South African bord-and-pillar workings.
- A field trial is required to prove and evaluate the bio-technology under mining conditions.

Methane-oxidising micro-organisms

The organisms that would be used occur naturally in mines and, in fact, are generally cultivated from samples of mine water. These micro-organisms obtain energy by oxidation of methane producing carbon dioxide and water as end products.

Health and safety

The introduction of a biological system may generate concerns regarding the potential risks of pathogenic (disease causing) bacteria. However, the risks associated with the use of methane-oxidising micro-organisms are considered to be negligible. Methane-oxidising bacteria are harmless, non-pathogenic (Group 1) micro-organisms.

There is generally no compulsory requirement for personal protective clothing and equipment for use with Group 1 micro-organisms. Nevertheless, it is advised that precautionary measures are adopted consistent with good international practice.

Proposed method of application

The proposed method of treatment would involve the installation of suspended bio-curtains at selected locations prior to sealing off an area. The bio-curtains would consist of a carbon cloth coated with immobilised micro-organisms. They would be activated by spraying with water and nutrients after installation. The bio-curtains should be protected during transport underground to prevent contact with handlers and also to guard them from accidental damage or contamination.

Due to the impracticality of treating extensive abandoned areas it is suggested that treatment is concentrated in a specifically selected area. The identified area may be disturbed ground or a faulted region in which significant gas emissions were detected during development. Alternatively, the treated area could comprise a selected number of roadways immediately inbye of the stoppings. Thus, an “inert” barrier would be created between the working mine and the sealed area.

The biological system may be required to remain effective for a decade or more. A spray system is therefore needed through which water, nutrients and fresh culture can be introduced. Remote monitoring of gas concentrations in the sealed area will indicate when replenishment is required and will also confirm the effectiveness of the installation.

Basic calculations indicate that some 11 bio-curtains, each of 9m² cross-sectional area should be capable of treating a methane emission rate of 5 litres/min per 300m³ of void volume.

A pilot trial

A suggested site for a pilot trial would consist of two, recently driven adjacent blind coal headings of similar length that could be temporarily, but securely sealed for the duration of the experiment. Bio-curtains would be installed in one of the headings. The other would act as a control. Gas concentrations in each heading would be remotely monitored using a simple tube bundle system. A pilot trial of some 12 months duration should be sufficient to evaluate the practical applicability of the technology.

Recommended safety precautions

- Method statements should be prepared describing procedures for handling, storage, treatment and salvage of biological materials at the mine in both surface and underground operations.
- Before introducing the bio-curtain system underground, the staff and workforce should be briefed on the precautionary measures to be adopted.
- Good hygiene practices should be maintained i.e. no eating and drinking while handling any microbial cultures.
- Gloves and safety spectacles should be worn when working with biological materials.
- Areas in which micro-organisms are being installed should be restricted access and biohazard signs displayed.
- On completion of the trial all the biological materials should be removed from the mine and disposed of to an appropriate licensed facility.
- Advice should be sought from a specialist company until the necessary biohazard expertise is gained by an appointed safety official at the colliery.

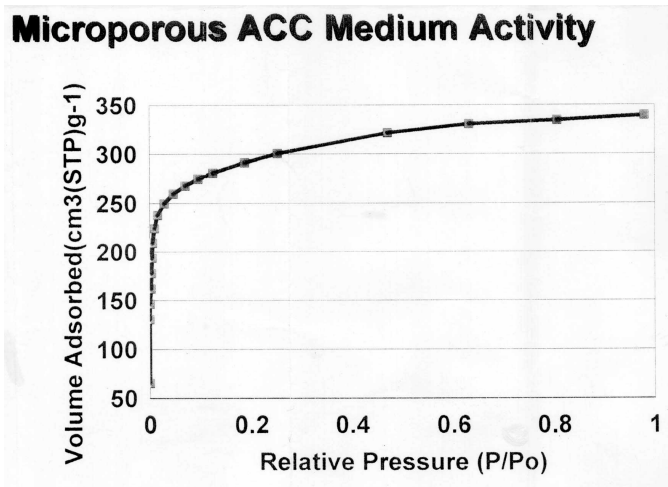


Figure 1. Adsorptive properties of activated charcoal cloth

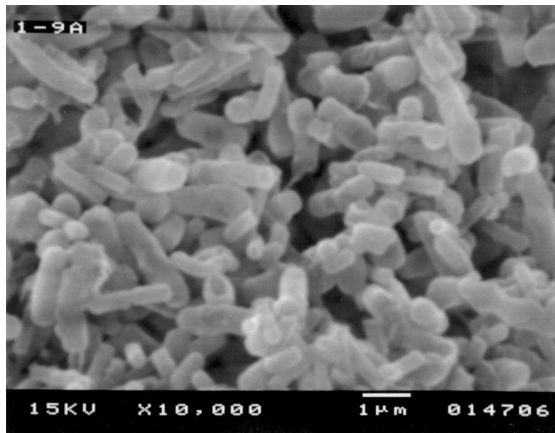


Figure 2. Photomicrograph showing colony of micro-organisms

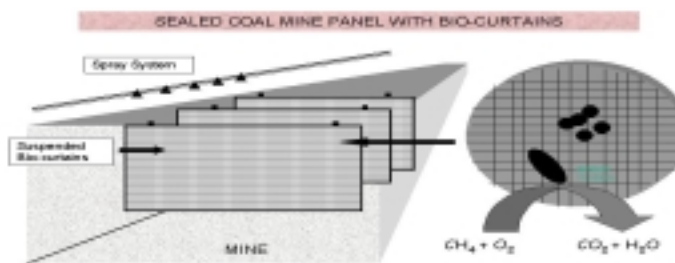


Figure 3. Proposed bio-curtain and spray irrigation system