MONITORING, EVALUATION AND VERIFICATION OF LONG-TERM PERFORMANCE OF THE PASSIVE WATER TREATMENT PLANT AT VRYHEID CORONATION COLLIERY

Report to the
Water Research Commission

by

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Monitoring, Evaluation and Verification of Long-term Performance of the Passive Water Treatment Plants at Vryheid Coronation Colliery

and

Continued Evaluation of the Integrated Managed Passive (IMPI) treatment system, Long-term monitoring of VCC passive treatment plant and three dimensional characterisation of sulfate reducing units

The financing of the project by the Water Research Commission and the contribution of the members of the Steering Committee are gratefully acknowledged.

The Steering Committee responsible for this project consisted of the following persons:

Mr HM du Plessis	Water Research Commission (Chairman)
Mr Nico Dooge	Xstrata Coal
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EXECUTIVE SUMMARY

BACKGROUND AND INTRODUCTION

There is an increasing demand for inexpensive, environmental friendly technologies to remediate acid mine drainage (AMD) and passive treatment technology is one of the key groups of processes that have been developed to address this need. Passive treatment technology is defined as a "water treatment system utilizing naturally available energy sources such as microbial metabolic energy, photosynthesis, chemical energy, natural topographical gradients to regulate flow and requires regular but infrequent maintenance".

Pulles Howard and de Lange Inc. (PHD) have undertaken a major research effort that started in 1994, in developing the patented Integrated Managed Passive (IMPI) treatment system. This technology can reliably remove sulfate, acidity and metals from AMD. The essence of the IMPI process is the subdivision of the overall treatment process into individual units, each designed and optimised to perform a key function. A key feature of this technology is that it will generally be required to operate for a number of years, typically decades, and continue to perform in accordance with its specified design criteria. However, research undertaken to date has shown that passive treatment plants do undergo a change in performance with time. Specifically, the plants operate at a high efficiency for the first 6-9 months before settling down to a lower but more sustainable efficiency over the long-term.

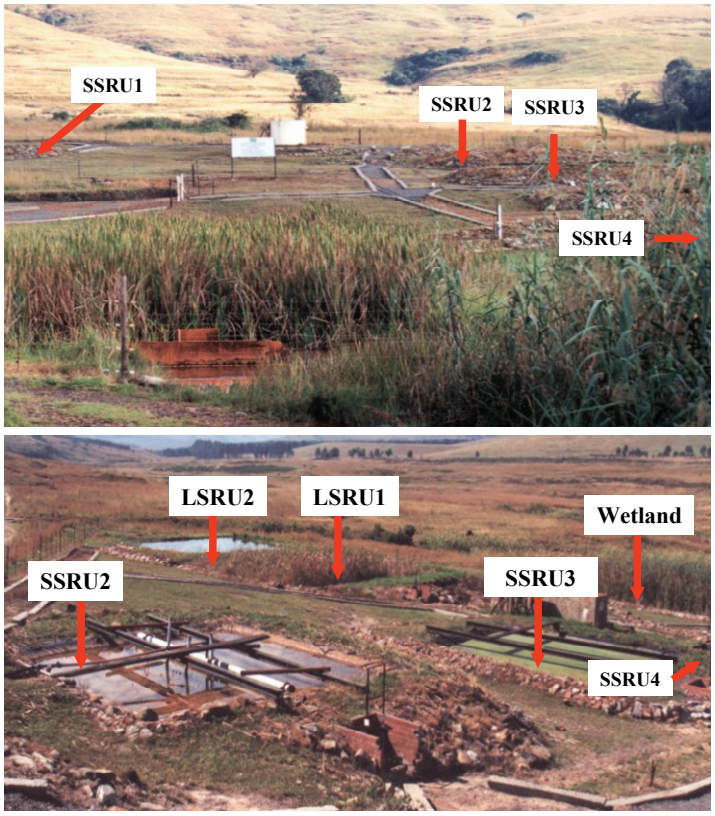
The success of the IMPI technology, compared to other passive treatment systems is attributed to the enhanced sulfate reduction performance of the Degrading Packed Bed Reactor (DPBR) also patented by PHD. The DPBR is packed with multiple layers of selected carbon sources (electron donors) available in the surrounding area and also receives regular inputs of a small dosage of readily available "kick-start" carbon such as liquid molasses in the feed. In the DPBR, feed water containing high sulfate and heavy metals is rapidly conditioned by removing dissolved oxygen to establish the desired redox potential of –300 mV. At this redox potential, sulfate reduction occurs, producing sulfide and alkalinity necessary for the hydrolysis of lignocellulose (LC), and the production of volatile fatty acids (VFA) which are utilised in enhanced sulfate reduction in the DPBR and downstream. Furthermore, metals are precipitated as metal sulfides. The effluent from this reactor contains reduced concentrations of metals and sulfate, and elevated sulfide, alkalinity and VFAs.

The performance of the IMPI systems can best be evaluated by studying the effective removal of sulfate, and the production of alkalinity and sulfide. The average sulfate load removal rate for the DPBR kinetic column reactors operated in the laboratory was 400 g sulfate removed/m^3 of carbon/day with a range of between 170 and 925, depending on the loading rate applied to the reactor. These values exceed the original target of 60 g sulfate removed/ m^3 of carbon/day set for the system to be economically viable. It must be noted that the international standard for "successful" sulfate load removal is in the order of only 30 g/m^3 carbon/d.

A pilot plant for the passive treatment of acid mine drainage (AMD) was designed, constructed and operated at Vryheid Coronation Colliery (VCC) by Pulles Howard & de Lange Incorporated (PHD) since 1996 and has contributed to major breakthroughs in passive treatment development. Based on discussions with researchers in the USA and UK, the VCC pilot plant reactors are believed to be the longest running, most closely monitored passive sulfate removal reactors in the world. These reactors,

which were monitored on a daily basis for nine years, followed typical trends with regards to passive sulfate reduction where initial sulfate reduction rates were high (coinciding with the availability of readily degradable carbon) and thereafter declining as only the more recalcitrant carbon is left.

When commissioned in 1996, the VCC pilot plant comprised of six sulfate reducing units (SRUs). Four of these were small sulfate reduction reactors (SSRUs), two were large sulfate reduction reactors (LSRUs) and a post treatment "polishing" facility comprising of an aerobic wetland and oxidation cascade as shown in the Figure below.



Initially, the average sulfate load removal (g sulfate/m^3 carbon/d) in the SRUs ranged between 27.9 (1996 to 1998) and 29.7 (1998 to 2001) after which it dropped to a constant but lower range between 15.7 and 18.9 (2002). In the verification of the long-term performance of the pilot plant, performance enhancement strategies developed in the DACST (department of Arts, Culture, Science & Technology) Innovation Fund project were implemented. These included the pilot scale application of the DPBR principles on the reactors already in operation for nine years. Three SSRUs (SSRU2, SSRU3 and SSRU4) were selected for this purpose. SSRU2 and SSRU4 were supplemented with a readily degradable carbon source in the form of liquid molasses at 0.1% dilution into the influent. After a period of 18-months operation, molasses supplementation into SSRU4 was terminated.

SSRU3 was decommissioned and a post-mortem analysis was undertaken after which a DPBR was then constructed and commissioned in its place. The DPBR was also dosed with molasses similar to SSRU2 and SSRU4. The DPBR was packed with similar carbon sources previously packed into SSRU3 for comparison reasons. The results of this study showed that the DPBR attained an average sulfate load removal of 85.0 g/m^3 carbon/d which was lower than that achieved during the DACST project in kinetic column reactors (averaging 400 g/m^3 carbon/d), but much higher than the average

sulfate load removed in all the other SSRUs. The difference in rates was attributed to the fact that kinetic column studies were not optimised for passive treatment system's site specific scenarios and scale-up. Although the DPBR outperformed SSRU1 there were comparable performances in the two systems attributed to the availability of readily degradable carbon present in both units over the same period scale. However, the performance of SSRU1 has since decreased, while the DPBR continues to reduce sulfate, remove metals and generate sulfide and alkalinity.

The supplementation of molasses to SSRU2 and SSRU4 led to increased sulfate load removal of 48.2 g/m^3 carbon/d (337%) and 49.5 g/m^3 carbon/d (399%) respectively. Termination of molasses supplementation to SSRU4 resulted in a decrease in sulfate load removal, generation of alkalinity and sulfide.

The implementation of the IMPI process performance enhancement strategies to the VCC pilot plant resulted in enhanced sulfate reduction compared to the older SSRUs indicating that the DPBR technology has moved the sulfate reduction process to a higher level. The use of the readily degradable "kick-start" carbon source (molasses) also indicated that it was effective in increasing sulfate reduction for the older sulfate reduction units that had much lower sulfate reduction efficiency.

Without the implemented performance enhancement strategies, none of the SRUs were able to achieve the target sulfate load removal of 60 g/m^3 carbon/d required in order for the technology to be economically feasible and had undergone a significant downward shift in their operating efficiency. However, these SRUs could not be summarily shut down without a thorough decommissioning and autopsy programme as it is critical to establish the cause of this shift. Detailed chemical and microbiological characterisation of the different carbon layers of the SRUs enabled the microbial identification of areas where active sulfate reduction occurred, as well as investigation of the degradation rate of the different carbon layers and on the hydraulics of the system.

This project, which is a combination of the research project K5/1623 and a consulting contract K8/578, reports on the monitoring and evaluation of the standard reactors and the reactors operating after implementation of performance enhancement strategies and the objectives of these studies are as follows:

1. Verify the long-term performance of the DPBR and SRUs modified with performance enhancement strategies at VCC for the removal of sulfate, metals and acidity.
2. Perform tracer studies on the DPBR.
3. Characterisation of the SRUs operated for nine years without any enhancement strategies in order to obtain a detailed chemical and microbiological (DNA extraction and molecular typing of microbial population) characterisation of the SRUs to establish and confirm the cause of system failure.

Although the VCC Passive treatment pilot plant has been in operation since 1996, the duration of this research project was only for a year, but together with the consulting project, the evaluation and monitoring of the plant data is presented in this report for the period October 2002 to March 2006 during which the performance enhancement strategies were implemented.

In order to prove that the strategies implemented enhanced the performance of the SRUs and that the DPBR does not undergo the decrease in performance, the following steps were followed:

1. Compare the DPBR performance against that of a nine year old SRU over the same period of operation after the respective commissioning. SSRU1 was selected as it closely resembled the operation of the DPBR *e.g.* both have the same volume of carbon, no bulking agent and have received a direct mixture of West and South Adit AMD since their commissioning. No molasses supplementation occurred in SSRU1 and sulfate reduction which could be attributed to molasses supplementation in the DPBR was also deducted from total sulfate reduction and converted back to sulfate load reduced for comparison reasons.
2. Compare performance of the SRUs prior to, during and after molasses supplementation. SSRU2 and SSRU4 were supplemented with molasses and after 18 months, supplementation to SSRU4 was terminated.

DISCUSSION OF RESULTS

The Table below summarises the average sulfate concentration removed and the average sulfate load removed of the different units. The DPBR was the only SRU achieving the target sulfate load removed of 60 g/m^3 carbon /d. Although the DPBR was supplemented with molasses, supplementation only contributed an average COD concentration of 71.0 mg/ℓ in the influent to the DPBR while the average concentration of sulfate reduced was 474 mg/ℓ. Molasses could therefore not be the only carbon source responsible for driving sulfate reduction and molasses was probably responsible for the enhancement of ligno-cellulose degradation, which sustained the reduction of sulfate.

Although the DPBR achieved the target sulfate load removed of 60 g/m^3 carbon /d, the effluent sulfate concentrations were still high – averaging 1089 mg/ℓ. It must be kept in mind that the optimum conditions for sulfate reduction did not exist for the DPBR and that sulfate reduction would be higher if the right operating conditions were selected through kinetic column studies.

The supplementation of molasses to SSRU2 and SSRU4 led to increased sulfate loads removed with SSRU2 averaging 45.2 g/m^3 carbon /d and SSRU4 40.2 g/m^3 carbon /d.

Unit	Period	Sulfate concentration reduced (mg/ℓ)	Sulfate load reduced (g/m^3 carbon/d)
SSRU1 – Standard	17/10/2002-09/03/2006	71.4	22.3
SSRU2 – Standard	17/10/2004-31/03/2003	Added	6.3
SSRU2 – Molasses	01/04/2003-09/03/2006	252	45.2
DPBR – Standard	17/10/2004-31/03/2005	806	68.6
DPBR – Molasses	01/04/2005-09/03/2006	420	76.5
SSRU4 – Standard	17/10/2004-31/10/2005	12.9	12.4
SSRU4 – Molasses	01/11/2005-11/10/2005	195	40.2
SSRU4 – No molasses	12/10/2005-09/03/2006	164	46.5
LSRU1 – Standard	17/10/2002-09/03/2006	60.8	7.3
LSRU2 – Standard	17/10/2002-09/03/2006	108	11.2

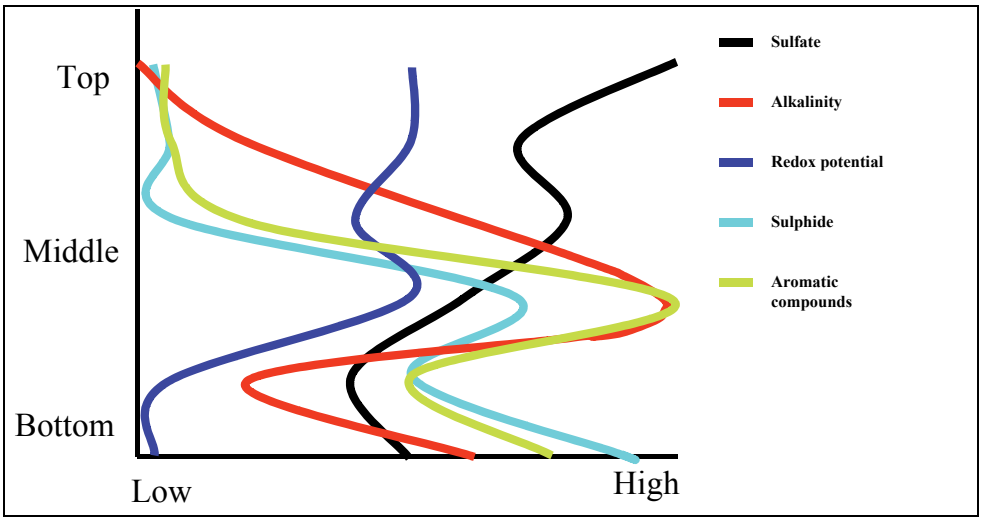
Hydraulic performance of the sulfate reducing units

Sodium bromide was successfully used as a tracer to generate tracer response curves. It is evident from the tracer response curve of the DPBR that some short-circuiting occurs within the DPBR.

However, the bromide peak recorded 61 h after tracer injection, coincided very well with the theoretical retention time of 68 h. A lag period was however involved with residual bromide concentrations being detected well after the theoretical response curve. The tracer response curve observed for SSRU2, which has been operational since 1996 indicated that hydraulically limiting areas exist within this bioreactor leading to a prolonged bromide response curve.

Three dimensional characterisation of SSRU1

The SRU sampling operation performed in October 2006 was executed successfully and, with the exception of the sawdust layers, it proved possible to identify all the other packing layers from the construction plans. Both water samples and a reactor core sample were recovered. Based on analysis of these, it is possible to draw provisional conclusions relating to the function and possibly also the failure of the conventional SRU packed bed reactor. These observations are considerably strengthened when compared to the studies of DPBR studies undertaken previously as controlled laboratory studies at PHD's carbon facility. The Figure below summarises the trend from top (influent) to bottom (effluent) of the more important parameters evaluated for SSRU1.



Previous attempts to characterise the sulfate reducing units (SRUs), in terms of the liquid fraction, at VCC proved to be very difficult. One of the major drawbacks from characterising the units is the creation of short-circuits within the units after sampling. Only liquid samples were drawn during the earlier study performed in 1998 and no detailed chemical analyses were performed on solid carbon material. Plastic pipes were forced through the carbon and were later sealed with cement. These studies, did however, find that oxic conditions prevailed in the bulk of the units. The presence of oxygen in the units indicated the lack of significant microbial activity, since the latter would have consumed the oxygen during biodegradation of organic matter. From this it was concluded that the biodegradation of the carbon sources in the systems was the rate limiting step.

While it was anticipated that a substantial portion of the lignocellulose complex of the woodchip fraction would remain present in the system, even after 9 years of reactor operation, it was surprising to note that even the soluble wood extractives fraction still remained as a substantial component of the woodchip sample. Together with the high residual levels of pectin observed, this indicates extremely poor utilization of the substrate and lignocellulose degradation. These observations are largely

supported by the SEM study. It should also be noted that wood particle size plays a role, and that sawdust was more degraded than woodchips over the course of reactor operation.

The SEM results of the woodchip samples did, however, show some differentiation between the Top, Middle and Bottom layers, which was also generally supported by the water sample analyses. It was apparent that the upper layer was more degraded than the woodchip layers lower down in the reactor. Consumption of aromatic compounds, TOC and VFA was also apparently higher in the upper layer. Elevated reducing sugars in this zone also suggested enhanced breakdown of the lignocellulose complex here.

The above results are in agreement with the Degrading Packed Bed Reactor (DPBR) hypothesis which argues that high activity in the upper layer is responsible for sustaining the activity of the full column. However when the easily available substrate for sulfate reduction becomes exhausted in this zone, the whole process gradually unwinds. This corresponds with the trend that emerged and accounts, in part, for the poor performance observed.

This phylogenetic study performed on SSRU1 at VCC provides a very interesting observation of the presence of many of the bacterial physiological types that have also been associated with the successful performance of the DPBRs. Studies, undertaken under controlled laboratory conditions on the DPBRs, have provided a structured outline of the microbial processes occurring in these systems. The observation of comparability of the two results provides a very useful indication that the failure of the SRU system was not due to the lack of appropriate biocatalytic genetic potential. Most of the necessary forms of bacteria found in the laboratory studies performed on DPBRs were present in SSRU1. However, a readily supply of biodegradable carbon is required to drive the process over the depth of the SRU and enhance lignocellulose degradation.

The results reported here provide an important supportive contribution to the operational hypothesis on which the DPBR reactor design concept is based.

Specialised studies performed on the solid carbon samples are very expensive and not all of the analyses performed proved to be valuable in evaluating the performance of the SRUs. Accurate oxygen determinations also proved to be difficult to measure under anaerobic conditions.

CONCLUSIONS

Long term performance of sulfate reducing units at VCC

During the operation of a DPBR, readily available carbon or kick-start carbon is fed to the system. This conditions the water by initiating anaerobic metabolic processes removing oxygen from the system and establishing the right redox potentials. Sulfate reduction starts adding sulfides and alkalinity to the system. The presence of high concentrations of sulfides and alkalinity prevents the re-polymerisation of complex carbon such as lignocellulose thereby enhancing the degradation thereof.

The commissioned DPBR has been operating for a period of 3 years and 5 months. Thus far, it has shown a constant and high sulfate reduction activity compared to the standard SRUs. The average sulfate load removed from the DPBR was 76.5 g/m^3 carbon/d. The DPBR was however not packed in

accordance with the implementation of the IMPI Process where kinetic column studies would normally be undertaken first with the results then being used to optimise the process according to site-specific parameters.

Molasses supplementation to SSRU2 and SSRU4 has led to an increase in sulfate reduction and sulfide production. The sulfate load removed for SSRU2 increased from 6.3 g/m^3 carbon/d (17/10/2004-31/03/2005) to 45.2 g/m^3 carbon/d after molasses supplementation (01/04/2005-09/03/2006). This represents a 316% improvement in sulfate load removal. The sulfate load removed in SSRU4 increased from 12.4 g/m^3 carbon/d (17/10/2004-31/03/2005) to 40.2 g/m^3 carbon/d after molasses supplementation. This represents an improvement of 324% in sulfate load reduced.

Enhanced sulfate reduction was maintained for a period of 3 years in SSRU2 and for a period of 28 months in SSRU4. The DPBR was successfully maintaining high sulfate loads removed for a period of 3 years and 5 months. The enhancement strategies were therefore implemented with success at the VCC Passive Treatment Pilot Plant.

Hydraulic performance of the sulfate reducing units

The following conclusions can be made from the tracer studies:

- Sodium Bromide was successfully used as tracer to generate tracer response curves.
- With regards to the DPBR:
  - Some short-circuiting occurred within the bioreactor
  - Hydraulically dead spaces occur leading to prolonged retention of the water in the DPBR
  - The bromide peak corresponded well with the theoretical retention time of 68 h.
  - Hydraulic configuration seems to be close to optimum as can be seen by the high sulfate reduction values
- With regards to SSRU2:
  - Hydraulically dead spaces occur leading to prolonged retention of the water in the SSRU2 unit.

The hydraulic configuration used in the DPBR has enabled an optimum diffusion of mine water throughout the reactors as can be seen by the high sulfate reduction values.

Three dimensional characterisation of SSRU1

The following conclusions were made:

- Even after 9 years of operation, the soluble wood extractives fraction still remained as a substantial component of the woodchip sample.
- Substrate utilisation was poor.
- Lignocellulose degradation was poor.

RECOMMENDATIONS

It is recommended that the DPBR and the SSRUs operating using the enhancement strategies are monitored further in order to determine the long-term performance of these units.

The implementation of the IMPI process, as patented after the Innovation Fund project, resulted in the desired result of enhanced sulfate reduction at the VCC pilot plant. When comparing the older VCC sulfate reduction units with the DPBR results it is clear that the new DPBR technology has moved the sulfate reduction process to a higher level. The use of the rapid breakdown kick-start carbon source (molasses) has also indicated that it is effective in increasing sulfate reduction for the older sulfate reduction units that were no longer achieving the required performance. The results of the DPBR and the kick start carbon supplementation at VCC have shown encouraging results that need to be monitored for at least another year.

It is recommended that the experimental programme as described in this report to perform tracer studies is used in future studies to determine the hydraulic performance of the SRUs.

Recommendations for future characterisation programmes include:

- The incorporating of dedicated sampling columns during the construction phase of the SRUs, which can be sealed off completely after sampling to prevent short-circuiting within the unit.
- Incorporation of thin perforated pipes within the SRUs to enable *in-situ* sampling over the depth of the column without actual disturbance of the SRUs.
- Focus on the following analysis as these proved to be the most valuable in assisting with the characterisation of the unit:
    - Water samples – sulfate, sulfide, alkalinity, aromatic compounds, volatile fatty acids and reducing sugars determinations.
    - Solid samples – Soluble pectin, lignocellulose fractions determinations as well as scanning electron microscopy studies.
    - Phylogenetic typing.

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ABBREVIATIONS

AMD	Acid mine drainage
AOAC	Association of Official Analytical Chemists
APHA	American Public Health Association
ARC	Agricultural Research Council
COD	Chemical oxygen demand
DACST	Department of Arts, Culture, Science and Technology
DPBR	Degrading packed bed reactor
EBRU	Environmental Biotechnology Research Unit
IMPI	Integrated Management Passive System
LSRU	Large sulphate reducing unit
PHD	Pulles Howard & de Lange (Pty) Ltd
SEM	Scanning electron microscope
SRU	Sulfate reducing unit
SSRU	Small sulfate reducing unit
TNC	Total non-structural carbohydrates
TOC	Total organic carbon
UK	United Kingdom
UN	University of Natal
USA	United States of America
VCC	Vryheid Coronation Colliery
VFA	Volatile fatty acids
WRC	Water Research Commission
WSC	Water soluble carbohydrates
XRD	X-ray diffraction
XRF	X-ray fluorescence

CHAPTER 1
INTRODUCTION AND BACKGROUND

1.1 INTRODUCTION

The salinisation of the public water system has been a subject of increasing concern in South Africa, and acid mine drainage (AMD) wastewaters have contributed substantially to this problem (Barnes and Romberg, 1986; Maree and Hill, 1989; Pulles *et al*., 1995; Scott, 1995; Gazea *et al*., 1996; Rose, *et al*., 1998; Younger, 2001; Johnson *et al*., 2002; Rose, 2002; Neba, 2006). Considerable research effort has been directed at process development for the remediation and treatment of these wastewaters (Maree *et al*., 1988; Lens *et al*., 2000; Whittington-Jones, 2000; Schoeman and Steyn, 2001; Rose, 2002; Neba, 2006). For this reason, biological systems have been the subject of particular interest given the need for sustainability over the long periods of time AMD flows are anticipated to require treatment (Rose *et al*., 1998; Chang *et al*., 2000; Johnson *et al*., 2002; Younger, 2001; Johnson and Hallberg, 2005; Neba, 2006). Both active (Rose, 2002, Neba, 2006) and passive treatment systems (Pulles *et al*., 1995; Younger *et al*., 1997; Younger, 1998; Zipper and Jage, 2001; Molwantwa *et al*., 2003; Younger, 2004; Coetser *et al*., 2005; Neba, 2006) have been developed for the removal of sulfate salinity and heavy metal contamination, and for neutralization of the acidic stream.

There is an increasing demand for inexpensive, environmental friendly technologies to remediate AMD. Passive treatment technology is defined as a "water treatment system utilizing naturally available energy sources such as microbial metabolic energy, photosynthesis, chemical energy, natural topographical gradients to regulate flow and requires regular but infrequent maintenance" (Pulles *et al*., 2003). Pulles Howard and de Lange Inc. (PHD) have undertaken a major research effort in developing the patented Integrated Managed PassIve (IMPI) treatment system that started in 1994. This technology can reliably remove sulfate, acidity and metals from AMD. The essence of the IMPI process is the subdivision of the overall treatment process into individual units, each designed and optimised to perform a key function (Coetser *et al.*, 2005).A key feature of this technology is that it will generally be required to operate for a number of years, typically decades, and continue to perform in accordance with its specified design criteria. However, research undertaken to date has shown that passive treatment plants do undergo a change in performance with time. Specifically, the plants operate at a high efficiency for the first 6-9 months before settling down to a lower but more sustainable efficiency over the long-term (Coetser, 2003a).

The success of the technology, compared to other passive treatment system is attributed to the enhanced sulfate reduction performance of the Degrading Packed Bed Reactor (DPBR) also patented by PHD. The DPBR is packed with multiple layers of selected carbon sources (electron donors) available in the surrounding area and also receives regular inputs of a small dosage of readily available "kick-start" carbon such as liquid molasses in the feed. In the DPBR, feed water containing high sulfate and heavy metals is rapidly conditioned by removing dissolved oxygen to establish the desired Redox potential of –300 mV. At this Redox potential, sulfate reduction occurs, producing sulfide and alkalinity necessary for the hydrolysis of lignocellulose (LC), and the production of volatile fatty acids (VFA) which are utilised in enhanced sulfate reduction in the DPBR and downstream. Furthermore, metals are precipitated as metal sulfides. The effluent from this reactor contains reduced concentrations of metals and sulfate, and elevated sulfide, alkalinity and VFAs.

The performance of the IMPI systems can best be evaluated by studying the effective removal of sulfate, and the production of alkalinity and sulfide. The average sulfate load removal rate for the DPBR kinetic column reactors operated in the laboratory was 400 g sulfate removed/m^3 of carbon/day with a range of between 170 and 925, depending on the loading rate applied to the reactor (Pulles *et al.*, 2003). These values exceed the original target of 60 g sulfate removed/ m^3 of carbon/day set for the system to be economically viable (Pulles *et al.*, 2003). Comparative sulfate reduction performance for the different phases of the research project is shown in Figure 1.1, where prior to the development of the DPBR, sulfate reduction levels ranged between 30 and 100 g/m^3 carbon/d. It must be noted that the international standard for "successful" sulfate load removal is in the order of only 30 g/m^3 carbon/d (*Pers. comm.* Pulles, 2004).

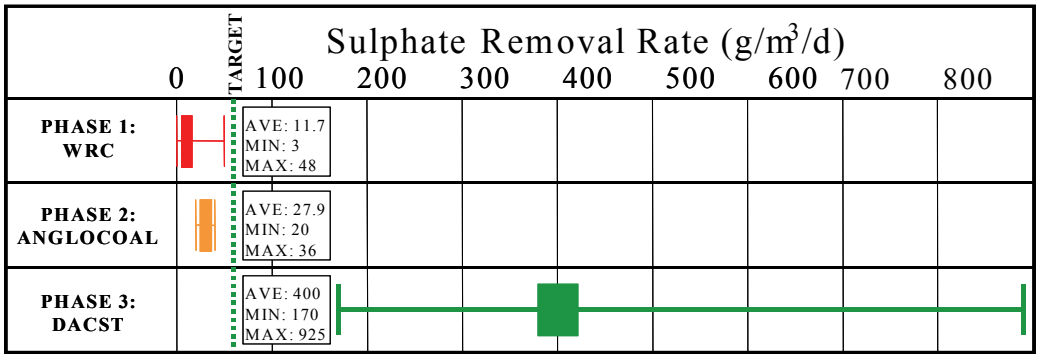


Figure 1.1: Summary of sulfate removal performance for all project phases to date

1.2 BACKGROUND: PILOT PLANT AT VRYHEID CORONATION COLLIERY (VCC)

A pilot plant for the passive treatment of acid mine drainage (AMD) was designed, constructed and operated at Vryheid Coronation Colliery (VCC) by PHD (Figure 1.2) since 1996 and has contributed to major breakthroughs in passive treatment development (PHD, 1999, Coetser *et al*., 2005).

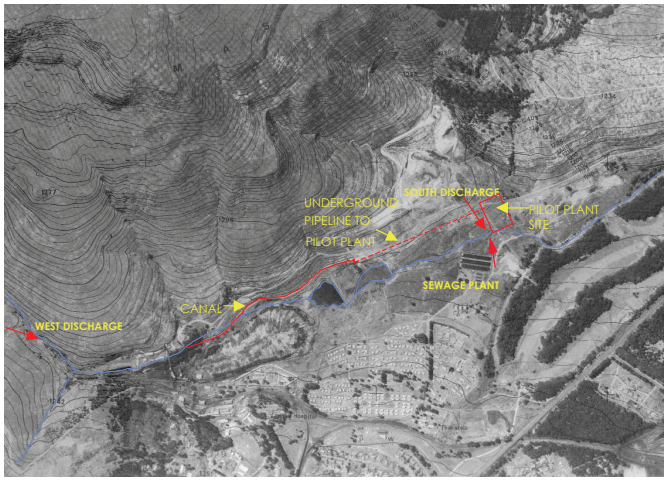


Figure 1.2: Location of Pilot Plant Site at Vryheid Coronation Colliery.

Based on discussions with researchers in the USA and UK, the VCC pilot plant reactors are believed to be the longest running, most closely monitored passive sulfate removal reactors in the world

(Pulles *Pers. Comm.*). These reactors, which were monitored on a daily basis for nine years, followed typical trends with regards to passive sulfate reduction where initial sulfate reduction rates were high (coinciding with the availability of readily degradable carbon) and thereafter declining as only the more recalcitrant carbon is left (Pulles *et al.,* 2003; Younger *et al.*, 2002; Coetser *et al.*, 2005).

When commissioned in 1996, the VCC pilot plant comprised of six sulfate reducing units (SRUs). Four of these were small sulfate reduction reactors (SSRUs), two were large sulfate reduction reactors (LSRUs) and a post treatment "polishing" facility comprising of an aerobic wetland and oxidation cascade (Figure 1.3). The wetland was constructed as a shallow reed-bed with a zigzag flow configuration and the oxidation cascade was constructed at an eight degree slope and layered with inert dolorite stone (Pulles *et al.*, 2003).

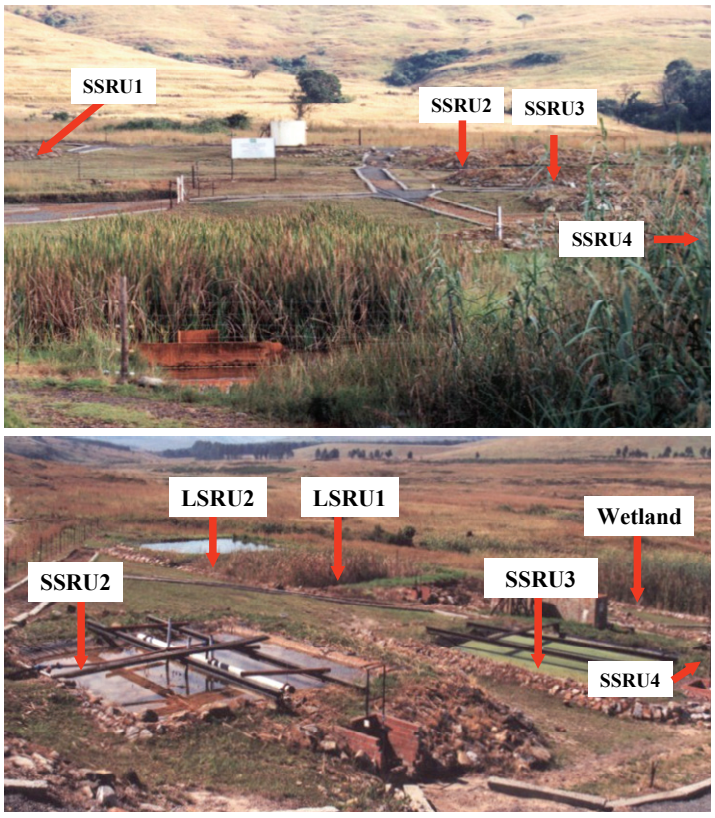


Figure 1.3: Photograph showing the VCC pilot plant.

The SRUs were constructed as inverted truncated pyramids, filled with layers of the various organic substrates including wood chips, grass cuttings, manure, chicken litter and sewage sludge. Of the SSRUs, SSRU1 and SSRU2 were down flow reactors while SSRU3 and SSRU4 were up flow reactors. All the SSRUs were constructed in series with SSRU1 flowing into SSRU2, then into SSRU3 and finally into SSRU4. SSRU4 was the only unit sealed on top using plastic sheeting to ensure anaerobic conditions. The two LSRUs running in parallel were designed to remove sulfates in a single stage reactor. LSRU1 was operated as a down flow unit, while LSRU2 was operated as an up flow unit. Effluent from the SRUs was then gravity fed to the post-treatment facility into the wetland through to the oxidation cascade prior to exiting into a small stream flowing outside the pilot plant.

Initially, the average sulfate load removal (g sulfate/m^3 carbon/d) in the SRUs ranged between 27.9 (1996 to 1998) and 29.7 (1998 to 2001) after which it dropped to a constant but lower range between 15.7 and 18.9 (2002). In the verification of the long-term performance of the pilot plant, in a WRC funded project (Project No.: 1348), performance enhancement strategies developed in the DACST Innovation Fund project were implemented (Pulles *et al.*, 2003; Coetser *et al.*, 2005). These included the pilot scale application of the DPBR principles on the reactors already in operation for nine years. Three SSRUs (SSRU2, SSRU3 and SSRU4) were selected for this purpose. SSRU2 and SSRU4 were supplemented with a readily degradable carbon source in the form of liquid molasses at 0.1% dilution into the influent. The liquid molasses dosing system was designed such that the preparation of molasses would be carried out every two weeks which fitted with the regular but infrequent maintenance defined by Pulles *et al.* (2003). After a period of 18-months operation, molasses supplementation into SSRU4 was terminated.

SSRU3 was decommissioned and a post-mortem analysis was undertaken after which a DPBR was then constructed and commissioned in its place (Coetser et al., 2005). The design of the DPBR was informed by findings of a study conducted by Wits University on the hydraulics of the SRUs. The findings of this study indicated that there were differences in vertical velocity of two orders of magnitude between the axis of the reactor and the outer zone (Fourie, 2002). This resulted in a large volume of substrate that was not being exposed to flow and therefore only the centre core of the reactor was hydraulically active (Fourie, 2002). As a result, the design of the DPBR was changed from the previous inverted pyramid to a rectangular design in order to maximise hydraulic activity (Coetser et al., 2005). The DPBR was also dosed with molasses similar to SSRU2 and SSRU4 (Coetser et al., 2005). The DPBR was packed with similar carbon sources previously packed into SSRU3 for comparison reasons. The results of this study (Coetser et al., 2005) showed that the DPBR attained an average sulfate load removal of 85.0 g/m^3 carbon/d which was lower than that achieved during the DACST project in kinetic column reactors (averaging 400 g/m^3 carbon/d), but much higher than the average sulfate load removed in all the other SSRUs. The difference in rates was attributed to the fact that kinetic column studies were not optimised for passive treatment system's site specific scenarios and scale-up. Although the DPBR outperformed SSRU1 there were comparable performances in the two systems attributed to the availability of readily degradable carbon present in both units over the same period scale. However, the performance of SSRU1 has since decreased, while the DPBR continues to reduce sulfate, remove metals and generate sulfide and alkalinity.

The supplementation of molasses to SSRU2 and SSRU4 led to increased sulfate load removal of 48.2 g/m^3 carbon/d (337%) and 49.5 g/m^3 carbon/d (399%) respectively. Termination of molasses supplementation to SSRU4 resulted in a decrease in sulfate load removal, generation of alkalinity and sulfide (Coetser *et al.*, 2005).

The implementation of the IMPI process performance enhancement strategies to the VCC pilot plant resulted in enhanced sulfate reduction compared to the older SSRUs indicating that the DPBR technology has moved the sulfate reduction process to a higher level. The use of the readily degradable "kick-start" carbon source (molasses) also indicated that it was effective in increasing sulfate reduction for the older sulfate reduction units that had much lower sulfate reduction efficiency.

Without the implemented performance enhancement strategies, none of the SRUs were able to achieve the target sulfate load removal of 60 g/m^3 carbon/d required in order for the technology to be economically feasible and had undergone a significant downward shift in their operating efficiency. However, these SRUs could not be summarily shut down without a thorough decommissioning and autopsy programme as it is critical to establish the cause of this shift. Detailed chemical and microbiological characterisation of the different carbon layers of the SRUs enabled the microbial identification of areas where active sulfate reduction occurred, as well as investigation of the degradation rate of the different carbon layers and on the hydraulics of the system.

1.3 OBJECTIVES

This project, which is a combination of the research project K5/1623 and a consulting contract K8/578, reports on the monitoring and evaluation of the standard reactors and the reactors operating after implementation of performance enhancement strategies and the objectives of these studies are as follows:

1. Verify the long-term performance of the DPBR and SRUs modified with performance enhancement strategies at VCC for the removal of sulfate, metals and acidity.
2. Perform tracer studies on the DPBR.
3. Characterisation of the SRUs operated for nine years without any enhancement strategies in order to obtain a detailed chemical and microbiological (DNA extraction and molecular typing of microbial population) characterisation of the SRUs to establish and confirm the cause of system failure.

Although the VCC Passive treatment pilot plant has been in operation since 1996, the duration of this study was only for a year, thus the evaluation and monitoring of the plant data is reported for the period October 2002 to March 2006 during which the performance enhancement strategies were implemented.

In order to prove that the strategies implemented enhanced the performance of the SRUs and that the DPBR does not undergo the decrease in performance, the following steps were followed:

1. Compare the DPBR performance against that of a nine year old SRU over the same period of operation after the respective commissioning. SSRU1 was selected as it closely resembled the operation of the DPBR *e.g.* both have the same volume of carbon, no bulking agent and have received a direct mixture of West and South Adit AMD since their commissioning. No molasses supplementation occurred in SSRU1 and sulfate reduction which could be attributed to molasses supplementation in the DPBR was also deducted from total sulfate reduction and converted back to sulfate load reduced for comparison reasons.
2. Compare performance of the SRUs prior- to, during and after molasses supplementation. SSRU2 and SSRU4 were supplemented with molasses and after 18 months, supplementation to SSRU4 was terminated.

CHAPTER 2
MATERIALS AND METHODS

2.1 LAYOUT AND CONFIGURATION OF THE VCC PILOT PLANT

Figure 2.1 illustrates the configuration of the VCC Pilot Plant as operated from November 1996 through to August 2002 (Appendix A), while the insert illustrates the plant modifications after performance enhancement strategies were implemented. This is the current state of the pilot plant and the details thereof have been described (WRC Report 1348; Coetser *et al*., 2005).

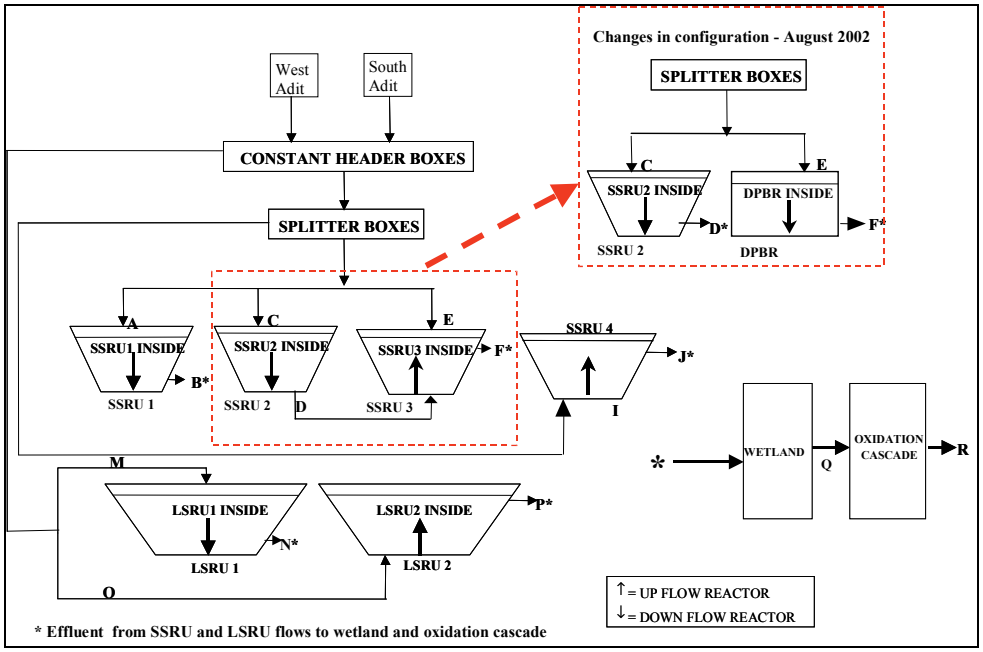


Figure 2.1: Schematic representation of the configuration of VCC pilot plant indicating sampling points and direction of flow (November 1996-August 2002)

The pilot plant currently consists of one standard SSRU (SSRU1), two modified SSRUs dosed with molasses (SSRU2 and SSRU4), a DPBR (former SSRU3) and two LSRUs (LSRU1 and LSRU2). The post treatment facility is comprised of the aerobic wetland and oxidation cascade. All units receive their feed water from the splitter boxes where the South- and West-Adit decants are mixed in a 1:1 ratio. Treated water from the SRUs and DPBR are “polished” in a post-treatment facility, consisting of a wetland and an oxidation cascade to remove residual nutrients added to the system (Figure 2.1). Effluent from the oxidation cascade is discharged into a natural stream flowing outside the pilot plant.

2.1.1 Acid Mine Drainage (AMD) Feed

West- and South-Adit seepage from the former VCC mine are mixed in a 1:1 ratio to form the feed of the SRUs. AMD from the West Adit is fed to a constant header box via a pipeline while seepage from the South Adit is pumped to a storage tank from where it is gravity fed to a constant header box (Figure 2.2). From each header box, South- and West-Adit water is gravity fed into separate compartments of the splitter boxes (Figure 2.2). Each of the SRUs is equipped with a splitter box to enable passive control of flow rate. Each splitter box has a V-notch in front and an overflow plate at

the back. Excess water flows over the overflow plate and a constant flow is obtained going through the V-notch into a mixing chamber after it is gravity fed through pipelines to the SRUs (Figure 2.2).

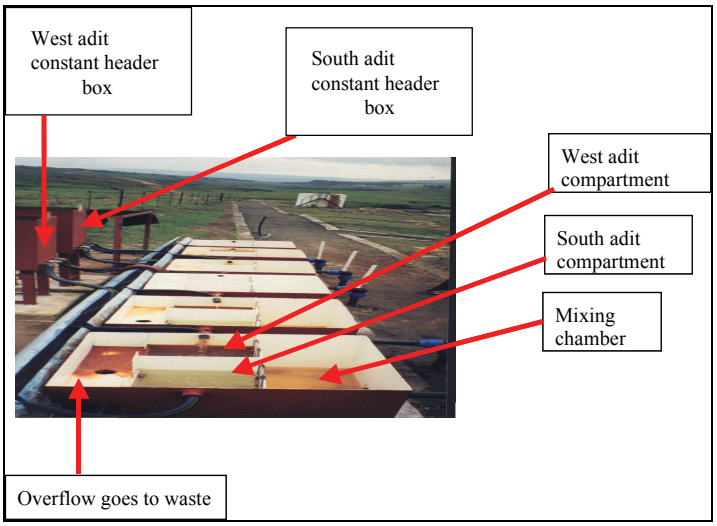


Figure 2.2: Photograph of splitter boxes at the VCC Pilot Plant showing the different compartments

2.1.2 Small Sulfate Reducing Units (SSRUs)

The SSRUs were packed with various layers of carbon (Table 2.1).

Table 2.1: SSRUs Carbon Substrate Layers (m^3)

Carbon Substrate (m^3)	SSRU1	SSRU2	DPBR	SSRU4
Hay	2 *(2 layers)*	None	4 *(4 layers)*	4 *(2 layers)*
Wood chips	6 *(3 layers)*	6 *(3 layers)*	4 (*3 layers*)	None
Sawdust	2 *(2 layers)*	2 *(2 layers)*	4 *(2 layers)*	6 *(2 layers)*
Sewage Sludge	2 *(2 layers)*	2 *(2 layers)*	Unknown	4 *(2 layers)*
Manure	4 (3 layers)	1 (1 layer)	2 *(2 layers)*	2 *(2 layers)*
Chicken litter	2 (2 layers)	None	2 *(2 layers)*	4 *(2 layers)*
Silage	None	7 *(3 layers)*	None	None
Bulking agent	None	None	None	8*(3 layers)*
Total Carbon	***18***	***18***	***18***	***20***

SSRU2 and the DPBR were supplemented with molasses at a concentration of 0.1% from the 1st of April 2003 while SSRU4 (Appendix B) was supplemented with molasses (0.1%) from the 1st of November 2003 (Coetser *et al*., 2005).

2.1.3 Large Sulfate Reducing Units (LSRUs)

The LSRUs were packed with various carbon sources as summarised in Table 2.2.

Table 2.2: LSRUs Carbon Substrate Layers

Carbon Substrate (m^3)	LSRU1	LSRU2
Hay	46 *(6 layers)*	50 *(6 layers)*
Wood chips	12 *(2 layers)*	20 *(3 layers)*
Sawdust	30 *(2 layers)*	20 *(3 layers)*
Sewage Sludge	12 *(2 layers)*	12 *(2 layers)*
Manure	30 *(2 layers)*	22 *(3 layers)*
Chicken litter	5 *(1 layers)*	10 *(2 layers)*
Bulking agent	121 *(8 layers)*	None
Total Carbon	***135***	***134***

2.1.4 Post Treatment Units

In the treatment process, nutrients and organic loading are added to the treated water, making it unsuitable for discharge without some form of post-treatment. In the aerobic wetland, organic nutrient load is removed while the oxidation cascade is responsible for the oxygenation of the treated water and the possible removal of manganese.

2.1.5 Operation of the VCC Pilot Plant

The operation of the pilot plant for the period 1996 to October 2002 is reported in WRC report No. 1348. For the purpose of this report, the evaluation and operation of the pilot plant is reported for the period October 2002 to March 2006. This study was carried out by comparing the DPBR with SSRU1 over the same period of operation after their respective commissioning periods. The current state of SSRU1 and the LSRUs was reported and compared to the DPBR and SSRU over the same period. SSRU2 and SSRU4 were compared prior to and during molasses supplementation. Furthermore, the effect of termination of molasses supplementation to SSRU4 was also analysed.

Flow rate, temperature, conductivity and pH are measured from Monday to Friday and recorded. Once a month, two sets of samples are collected at the pilot plant. One set is collected at 16 sampling points and the other set is collected as just above or below the carbon bed (inside samples) at SSRU1, SSRU2, SSRU3 and the LSRUs (Figure 2.1).

2.1.6 Hydraulic Loading Rates

Target flow rates for the pilot plant units are summarised in Table 2.3.

Table 2.3: Target flow rates (m^3/d) for the different SRUs for the period 17/10/2002 to 09/03/2006

Unit	Flow rate (m^3/d)	
	Period	
	17/10/2002-20/11/2003	21/11/2003-09/03/2006
SSRU1	1.72	2.77
SSRU2	1.72	2.96
DPBR	1.72	2.96
LSRU1	12.4	12.4
LSRU2	12.4	12.4

2.1.7 Sampling regime and data acquisition

A description of the various samples taken at different locations in the VCC pilot plant is given in Table 2.4.

Table 2.4: Descriptions of samples taken at different locations in the pilot plant

SAMPLING POINT	DESCRIPTION
A	SSRU1 IN
B	SSRU1 OUT
C	SSRU2 IN
D	SSRU2 OUT
E	DPBR IN
F	DPBR OUT
I	SSRU4 IN
J	SSRU4 OUT
M	LSRU1 IN
N	LSRU1 OUT
O	LSRU2 IN
P	LSRU2 OUT
Q	Oxidation Cascade IN
R	Oxidation Cascade OUT
WEST ADIT	Acid Influent AMD
SOUTH ADIT	Neutral Influent AMD
SSRU1 – Inside	Samples collected just below carbon layer (LSRU2) is an upflow unit. Samples collected just above the carbon layer (DPBR, SSRU1, 2 and LSRU1) are down flow units
SSRU2 – Inside	
DPBR – Inside	
LSRU1 – Inside	
LSRU2 – Inside	

The analyses carried out on the samples include:

- Sulfate concentration
- Sulfide concentration
- Alkalinity
- Chemical oxygen demand (COD)
- pH
- Redox potential
- Electrical conductivity
- Manganese concentration
- Iron concentration
- Aluminium concentration

The sampling procedure for the influent and effluent samples of the sulfate reducing units and post treatment facility is as follows:

Sulfate Concentration: A volume of 20 ml sample is preserved with 10 ml (1 *N*) HCl.

Sulfide Concentration: A volume of 50 ml sample is preserved with 4 drops zinc acetate (APHA, 1998).

Other Analyses: A 1 ℓ unpreserved sample filled to the brim is collected for the analysis of redox potential, pH, alkalinity and metal concentrations.

Inside samples are collected as a composite sample for each of the SRUs in a 1 ℓ volume, where five (SSRUs) and nine (LSRUs) sub-samples were taken at different points in the SRUs to make up the 1 ℓ composite sample. In the up flow reactor (LSRU2), the inside samples were collected just below the carbon layer in order to measure effects on treated water due to re-oxidation of sulfide to sulfate (Figure 2.1). In the down flow reactors (DPBR, SSRU1, SSRU2 and LSRU1), inside samples were collected just above the carbon layer to measure the effect of evaporation and rainfall on sulfate and sulfide concentrations on inflow water (Figure 2.1).

2.1.8 Data reporting

2.1.8.1 Sulfate load

An economic feasibility study performed by PHD (1999) indicated that in order for a passive treatment system to be economically viable, 60 g of sulfate must be removed per cubic metre of carbon per day (g/m^3 carbon/d). Sulfate load removed is calculated using the monthly influent and effluent sulfate concentrations and measured influent flow taking the effect of rainfall and evaporation into account according to the following equations:

$$\text{Sulfate load in } (g/m^3 \text{ carbon/d}) = \frac{[SO_4^{2-} \text{ concentration IN (mg/ℓ) x influent flow } (m^3/d)] + [SO_4^{2-} \text{ concentration in rain (mg/ℓ) x rainfall } (m^3/d)]}{\text{volume of carbon } (m^3)}$$

Sulfate load out (g/m^3 carbon/d) =

$$\frac{SO_4^{2-} \text{ concentration OUT (mg/ℓ) x [influent flow (m}^3\text{/d) + rainfall (m}^3\text{/d) – evaporation (m}^3\text{/d)]}}{\text{volume of carbon (m}^3\text{)}}$$

Sulfate load removed (g/m^3 carbon/d) = Sulfate load in (g/m^3 carbon/d) – Sulfate load out (g/m^3 carbon/d)

2.1.8.2 Average pH

The following calculations were used to determine the average pH:

1. Convert pH values to the activity of hydrogen (H^+) *e.g.* 10^(-pH)
2. Average the activity of the H^+ for the period investigated.
3. Convert the average activity to pH *e.g.* –log(average activity)

2.2 CHARACTERISATION OF SSRU1

In situ determinations of the following parameters were done at 250 mm intervals by lowering the probe slowly down the liquid sampling core:

- Electrical conductivity
- Total dissolved solids
- Salinity
- Dissolved oxygen
- pH
- Redox potential

Liquid and solid samples were collected and analysed as summarised in Tables 2.5 and 2.6. The detailed methods are outlined in Appendices C and D.

Table 2.5: Summary of chemical and physical analyses performed on the liquid samples

Responsible laboratory	Analysis	Method
PHD	Sulfate, Sulfide, COD, Alkalinity, Iron, Manganese, Aluminium, Calcium, Copper, Lead, Magnesium, Potassium, Sodium, Zinc	APHA,1998
Agricultural Research Council (ARC)	Starch	Glucose concentration of sample is determined and the values multiplied by the factor 0.912 in order to convert it to % starch
	Water soluble carbohydrates (WSC)	Carbohydrates measured spectrophotometrically against standards (Harris, 1990)
	Total non-structural carbohydrates (TNC)	Digestion of sample with amylase enzymes and carbohydrates measured spectrophotometrically against standards (Faichvey and White, 1983)
Environmental Biotechnology Research Unit (EBRU)	Aromatic compounds	Section 2.3.2.5.1
	Total organic carbon (TOC)	Section 2.3.2.5.2
	Volatile fatty acids (VFAs)	Section 2.3.2.5.3
	Reducing sugars	Section 2.3.2.5.4

Table 2.6: Summary of chemical analyses performed on the solid samples.

Laboratory	Analysis	Method
ARC	Aqua regia digest	APHA, 1998
	Moisture content	APHA, 1998
	Freeze dried	Vacuum Technologies (Pty(Ltd))
	Dry matter content	Samples are dried overnight at 100ºC to determine dry matter (Harris, 1990)
	Ash content	Ash content is measured after incineration overnight at 550ºC and then for 2 h at 900ºC (Harris, 1990)
	Total Kjeldahl nitrogen/Crude protein content	Crude protein can be estimated as Kjeldahl N x 6.25 using a LECO FP-2000 protein analyser (LECO AFRICA (Pty(Ltd))
	Crude fat	Crude fat is determined through ether extraction (AOAC, 1984)
	Crude Fibre content	% Crude fibre is calculated as follows: $\% \text{ Crude Fibre} = \frac{\text{RCD} - \text{RCA}}{\text{original sample mass}} \times 100$ where: RCD = residue in crucible after drying RCA = residue in crucible after ashing (AOAC, 1984).
	Lignin, cellulose and hemicellulose	The gravimetric method of Goering and van Soest (1970) is used to determine the concentration of hemicellulose, cellulose and lignin.
	In vitro digestibility	It involves a 48 hour fermentation by rumen micro-organisms in a buffer solution followed by a 48 hour pepsin digestion after acidifying with hydrochloric acid (Tilley and Terry; 1963).
Council for Geoscience	*X-ray diffraction analysis (XRD)	Section 2.3.2.7
University of the Witwatersrand	*X-ray fluorescence analysis (XRF) for major oxides (including wt% S and trace metals	Section 2.3.2.7
University of Fort Hare	*Mineralogy identification using microscopy	Section 2.3.2.7
EBRU	Soluble pectin	Section 2.3.2.5.5
	Lignocellulose fractions	Section 2.3.2.5.5
	Scanning electron microscopy (SEM) studies	Section 2.3.2.5.6
PHD	Water extraction and analysed for metals as listed for liquid samples	APHA, 1998
	Aqua regia digest leachate (Waterlab) analysed for metals as listed for liquid samples	APHA, 1998

*Used for mineralogical and geochemical analysis

2.3 HYDRAULIC PERFORMANCE OF THE SULFATE REDUCING UNITS (SRUS) AT THE VRYHEID CORONATION PASSIVE TREATMENT PLANT: TRACER STUDIES

Tracer studies (Appendix E) were carried out according to the University of Natal Pollution Research Group method outlined in Appendix F.

CHAPTER 3
EVALUATION OF PERFORMANCE ENHANCEMENT STRATEGIES

3.1 BACKGROUND

Several reports have been documented on the operation of the VCC passive treatment plant which has been in operation since 1996 (Pulles *et al.*, 2003; PHD, 2004; WRC report No.: 1348; Coetser *et al.*, 2005). The essence of these reports indicate that similar to all passive treatment systems, the VCC pilot-plant has gone through the initial high performance in terms of sulfate concentration and load removal followed by a lower but constant average sulfate concentration and load removal. This decrease in performance is reported to be in common with most passive treatment plants where initially, there is readily degradable carbon available for sulfate reduction (PHD, 2004; Coetser, 2005; Younger 2001). Once depleted, the remaining carbon (lignocellulose material) is not readily degradable by SRB resulting in a slow release of carbon from other reactions, which results in less carbon source for SRBs to carry out sulfate reduction, thus the sulfate load removal becomes lower.

In 2002, performance enhancement strategies were implemented by dosing of molasses to SSRU2 and SSRU4 and the decommissioning of SSRU3 and commissioning of a DPBR (Coetser *et al.*, 2005). This resulted in the standard SRU (SSRU1 and LSRUs, operated without the enhancement strategies) showing a lower performance compared to the SSRUs dosed with molasses. The performance of SSRU1 was even lower than that of the DPBR which was constructed differently and dosed with molasses after commissioning (Coetser *et al.*, 2005).

3.2 EVALUATION AND COMPARISON OF UNIT PERFORMANCE

The performance of the DPBR was compared to that of SSRU1 for the same time scale of operation (Months 6 to 39 after commissioning) and this is labeled DPBR and SSRU1 respectively. SSRU1-current, denotes the current state of SSRU1. The data for SSRU2 and SSRU4 is presented as pre-molasses and molasses (during supplementation) respectively. A post-molasses for SSRU4 is also reported for the period after termination of molasses supplementation (Appendix B). The data for LSRU1 and LSRU2 is presented for the current state of the LSRUs.

All data is presented as average values at a particular period of operation.

3.2.1 AMD Neutralization

The neutralization capability of the reactors was analysed in terms of pH and alkalinity production. The SRB derive energy from the carbon source to reduce sulfate, thereby producing sulfide and bicarbonate ions as products. The bicarbonate ions result in alkalinity and pH increase. These results are depicted in Figure 3.1.

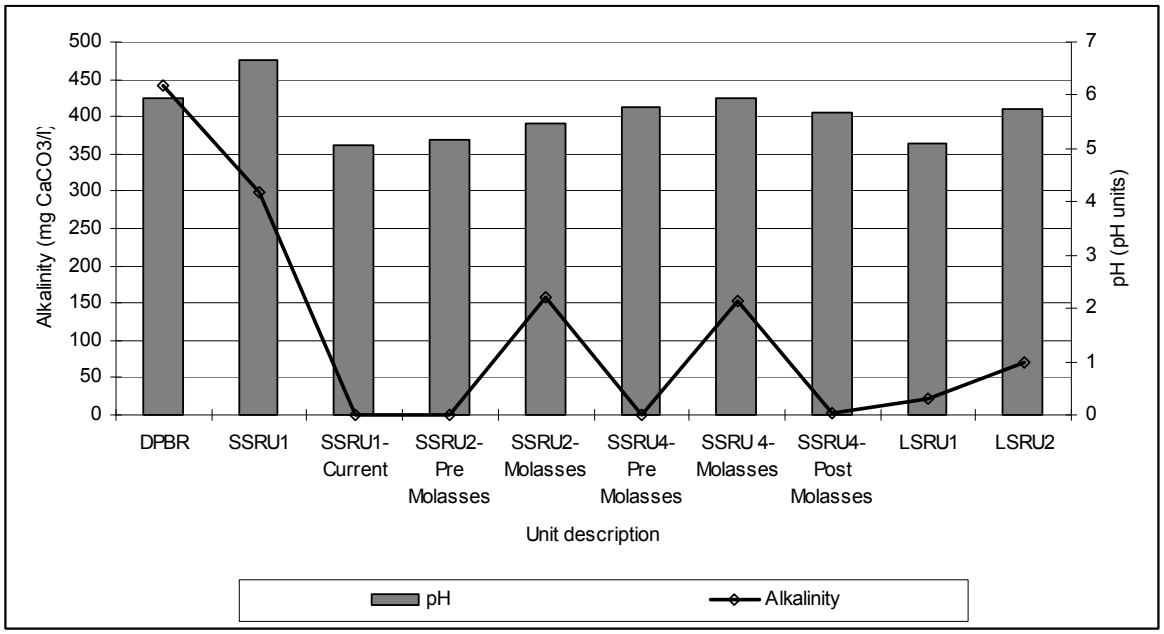


Figure 3.1: Average alkalinity generated (as mg $CaCO_3$/ℓ) and pH (pH units) data for the different units at the different states of operation for the period October 2002 to March 2006.

Although the DPBR had lower pH compared to SSRU1 over the same time scale of operation, the alkalinity generated by the DPBR (441 mg/ℓ) was higher than that of SSRU1 (298 mg/ℓ). The neutralization results of the DPBR and SSRU1 are comparable and this is attributed to the availability of readily degradable carbon in both systems after commissioning. However, after nine years of operation, the neutralization efficiency of SSRU1 has decreased with pH at 5.09 and there is no alkalinity generated. An increase in alkalinity generation and pH was also recorded for SSRU2 during molasses supplementation. The termination of molasses supplementation further decreased the neutralization efficiency of SSRU4 confirming that the dosing of readily degradable carbon revived and thus increased reactor performance. This increased performance is attributed to the enhanced sulfate reduction as a result of easily degradable carbon, resulting in sulfide and alkalinity generation which further catalyses lignocellulose degradation unlocking the carbon that has been recalcitrant and inaccessible to the SRBs. Thus further SRB activity occurs, and more sulfate load is removed. The LSRUs although bigger in size, are outperformed by the DPBR and SSRU1 over the same time scale, and SSRU2 and SSRU4 during molasses supplementation.

3.2.2 Sulfate

Figure 3.2 is a graphical presentation of the average sulfate concentration (mg/ℓ) and sulfate load (g/m^3 carbon/d) removed for the different units at the different states of operation.

The DPBR was the only SRU achieving the target sulfate load removal of 60 g/m^3 carbon /d with an average sulfate load removal of 78.6 g/m^3 carbon/d. This removal was lower compared to that achieved in the kinetic column studies (400 g/m^3/d). This was attributed to the fact that the kinetic column scale was smaller, synthetic mine water was used and the carbon sources used to pack the columns and the units were different.

As described earlier, the availability of readily degradable carbon to drive sulfate reduction during the first few months of operation in both the DPBR and SSRU1 over the same time scale led to the high initial sulfate removal. However, it is important to note that molasses supplementation enabled the continued high sulfate removal in the DPBR to overcome limited carbon availability through enhanced lignocellulose degradation.

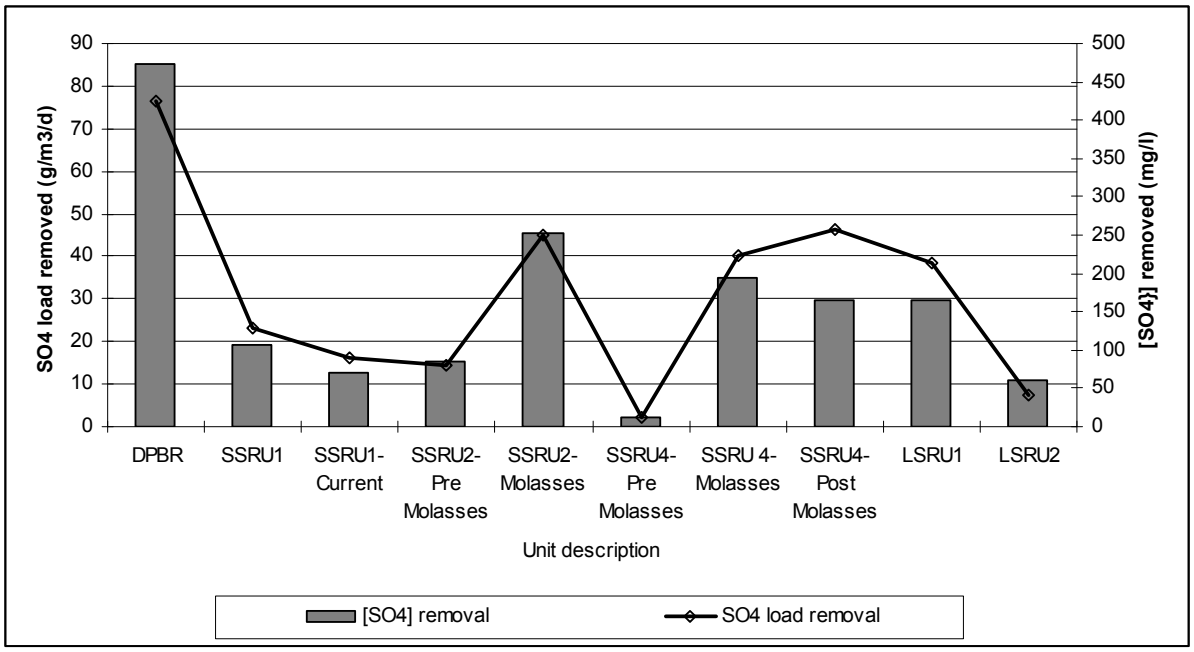


Figure 3.2: Average sulfate concentration (mg/ℓ) and sulfate load (g/m^3/d) removed for the different units at the different states of operation.

When comparing the sulfate concentration and sulfate load reduced between the DPBR and SSRU1 for the same period, the DPBR outperformed SSRU1 (76.5 versus 23.0 g/m^3 carbon/d). It is important to note that the continued performance over extended periods in terms of sulfate reduction is required. It is clear that after 9 years of operation, SSRU1 is not able to drive sulfate reduction to achieve the target sulfate load reduced. The hydraulic short-circuiting taking place in SSRU1 also contributed to the lower sulfate concentration removal.

When correcting the amount of sulfate reduced with the amount of sulfate reduced which could be attributed to molasses supplementation, it was found that very little sulfate reduction could be attributed to the molasses and that even when subtracting this contribution, there was significantly increased sulfate load removal. This is in accordance with earlier column and laboratory studies that indicated that the molasses acts as a catalyst in the hydrolysis of lignocellulose.

Molasses supplementation increased the sulfate load removal of SSRU2 and SSRU4 and the termination of molasses supplementation in SSRU4 led to a decrease in sulfate load removed

3.2.3 Sulfide production

Sulfide production correlated with sulfate reduction and alkalinity production observed (Figures 3.1, 3.2 and 3.3). Low sulfide production was observed prior to molasses supplementation in both SSRU2

and SSRU4 compared to the average sulfide produced during supplementation. The highest production of sulfide was recorded for the DPBR correlating with the high sulfate removal (Figure 3.2). Compared to the DPBR over the same time scale, SSRU1 had a lower average sulfide production (46.5 mg/ℓ versus 95.3 mg/ℓ). This coincides with the lower sulfate reduction observed in SSRU1 over the same period.

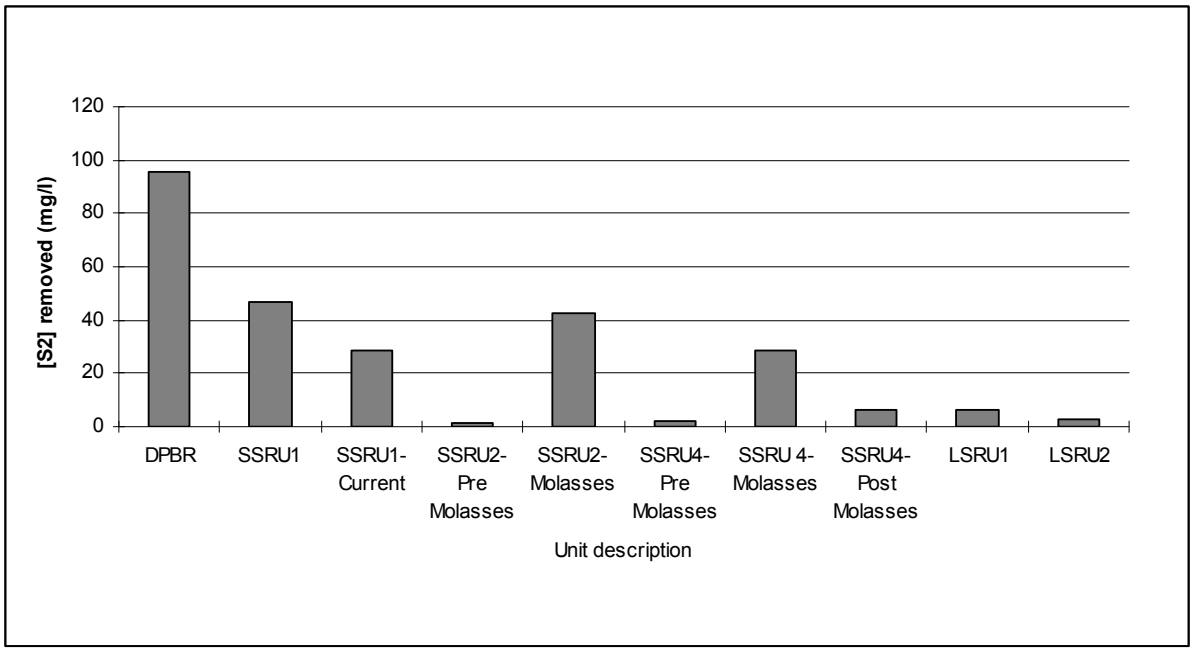


Figure 3.3: Average sulfide concentration (mg/ℓ) generated for the different units over the different states of operation.

3.2.4 Metal Removal

Of the three metals monitored, iron and aluminium removal is expected to occur while manganese removal is not expected at low pH. However, the highest manganese removal was recorded in the DPBR, averaging 61.3% (Figure 3.4). Molasses supplementation of SSRU2 and SSRU4 led to manganese removal where none was recorded prior to supplementation (Figure 3.4). The decrease in removal of manganese from SSRU4 after molasses supplementation was terminated further indicates that the there is a link between molasses supplementation and manganese removal.

There was more iron removal recorded for SSRU1 compared to the DPBR for the same time scale of operation. This could be explained by the fact that there was readily degradable carbon in both units driving active sulfate reduction and metal precipitation as metal sulfides. However, the result does not correlate with the lower sulfide recorded for SSRU1. The increase in iron average removal during molasses supplementation to SSRU2 and SSRU4 ranged between 60 and 80% respectively. Termination of molasses supplementation did not affect iron removal in SSRU4. Aluminium removal followed a similar trend to iron removal in all units. Currently, SSRU1 and the LSRUs have lower metal removal efficiency compared to the DPBR and SSRU2 supplemented with molasses.

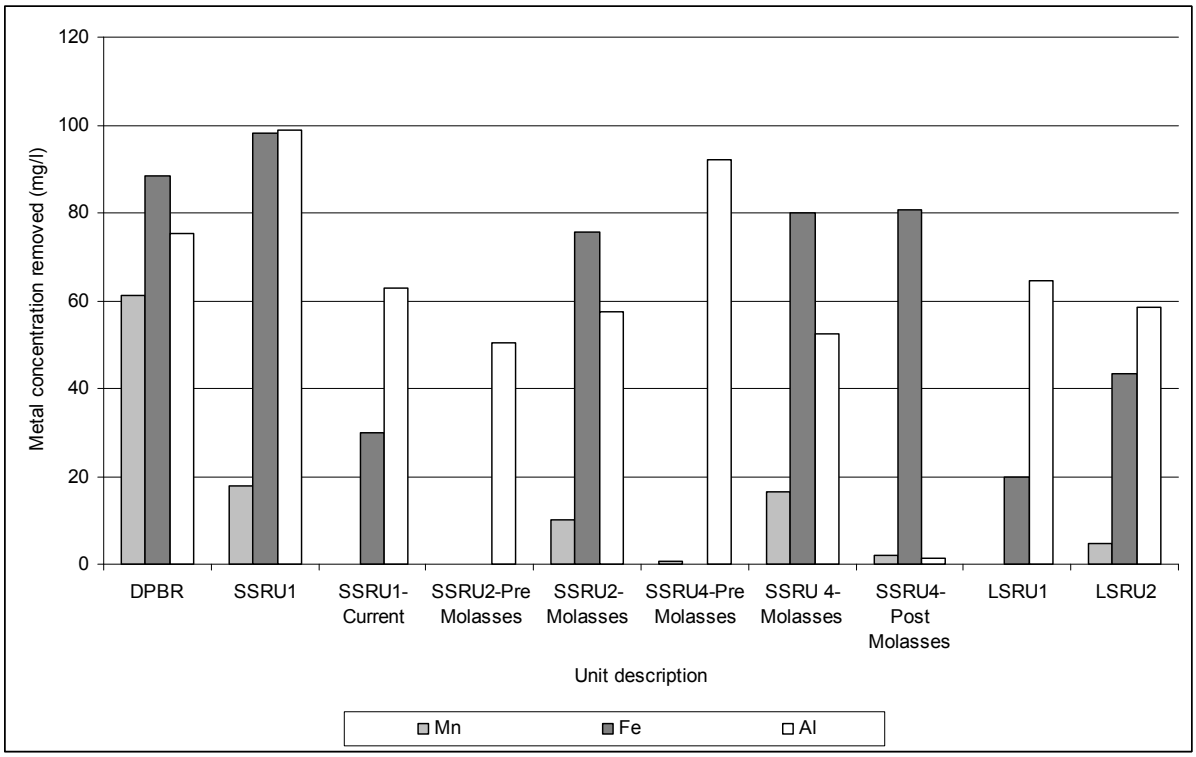


Figure 3.4: Average metal concentration removal (mg/ℓ) for the different units over the different states of operation.

3.3 CONCLUSIONS

The following conclusions were reached from this study on the monitoring and verification of the performance of the SRUs prior to and after performance enhancement strategies were implemented:

1. The lower sulfate reduction (pH, alkalinity and sulfide production, sulfate removal and metal removal) was observed in the SRUs prior to molasses supplementation and increased after supplementation. The decrease in performance recorded for SSRU4 after molasses supplementation was terminated confirms the previously identified catalytic function whereby the readily degradable carbon in the molasses unlocks the hydrolysis of recalcitrant carbon remaining in the lignocellulose even after years of operation. The LSRUs, although bigger in size and having been in operation for nine years, still have a lower performance compared to those supplemented with molasses and the DPBR.
2. Molasses supplementation resulted in increased sulfate reduction and overall performance of the SRUs indicating a potential for reviving poorly performing passive sulfate reduction system.
3. The DPBR performance when compared to SSRU1 performance over the same time scale was comparable as in both cases readily degradable carbon source was available for SRB to carry out active sulfate reduction. Of importance is the fact that the DPBR continues to outperform SSRU1 and over a longer period a clearer comparison can be made of the DPBR performance compared to that of SSRU1.
4. The DPBR achieved and maintained a sulfate load removal above the target 60 g/m^3 carbon/d which had not previously been achieved at the VCC pilot plant. Although lower than the level reached in kinetic column studies, the levels reached by the DPBR would be sufficient for the

technology to be economically viable. After 3 years and five months of operation, it could be conclusively confirmed that the DPBR had a higher sulfate load removal efficiency compared to standard SRUs irrespective of size.

5. The performance enhancement strategies implemented at the VCC pilot plant indicate that the DPBR principles and reactor design, play a vital role in the improved performance of the DPBR (in terms of sulfate and metal removal, and alkalinity and sulfide production) over standard SRUs. Furthermore, a higher sulfate load removal, sulfide and alkalinity production and metal removal was maintained in the DPBR system compared to the commonly reported performance of passive treatment systems where an initial high performance is followed by a decreased but constant performance in terms of sulfate removal.

CHAPTER 4
THREE DIMENSIONAL CHARACTERISATION OF SSRU1

4.1 INTRODUCTION

SSRU1 was selected for a three dimensional characterisation where both the liquid and solid samples were analysed in order to establish a three dimensional picture of the unit after nine years of operation. The sampling took place on the 17 to 19th of October 2005. The influent (a mixture of 1:1 ratio of West- and South-Adit AMD) to SSRU1 had the following characteristics (Average for the period 10-19 October 2006):

- pH = 5.88
- Electrical conductivity = 129 mS/m
- Sulfate concentration = 2174 mg/ℓ
- Manganese concentration = 8.80 mg/ℓ
- Aluminium concentration = 1.58 mg/ℓ
- Iron concentration = 0.84 mg/ℓ

4.2 MATERIALS AND METHODS

The detailed methodology for the three dimensional characterization is reported in Appendix C.

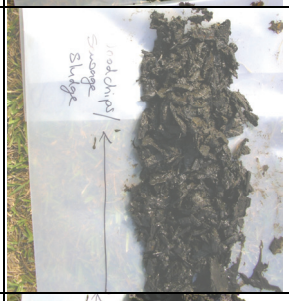
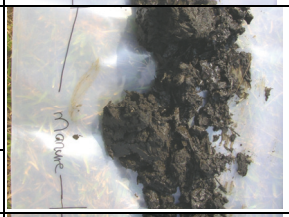
4.3 SAMPLE DESCRIPTION

SSRU1 was covered with a bright orange chemical sludge (Figure 4.1). After collection of the solid samples, careful inspection of the solid samples was made in an attempt to identify the particular packing materials used in each layer from Columns A and C. With the exception of the sawdust layer, it proved possible to correlate the original construction plan with the core samples recovered from Column A. It was therefore decided to use Column A for the chemical characterisation as some mixing of the carbon probably occurred during the coring of Column C. The sawdust samples were apparently completely degraded and presented as a soft paste.

Table 4.1 shows the numbering of samples drawn, including the auger sample number and the numbers of the samples used for chemical and molecular analysis performed by EBRU. The depth at which the different samples were taken is also indicated in Table 4.1. Table 4.1 also includes photos of the different layers of carbon as extracted at different depths of SSRU1, coinciding with the original carbon layers packed in 1996.

Table 4.1: SSRU1 sampling profile from Column A

Auger number	Chemical sample no.	Molecular sample no.	Depth	Characteristics	Photo of carbon at specific layer
1	A1	1	0-10	Chemical sludge	
1	A2	2	10-230	Sewage sludge	
1	A3	3	230-300	Chicken Litter	
1	A4	4	300	Sawdust	
2	A5	5	300-830	Woodchips	
2	A6		300-830	Manure	
3	A7	6	830-890	Hay/Chicken Litter	
3	A8		830-890	Manure	

Auger number	Chemical sample no.	Molecular sample no.	Depth	Characteristics	Photo of carbon at specific layer
4	A9	7	890-1130	Woodchips/ manure	
5	A10	8	1130-1170	Sawdust/ woodchips	
5	A11		1130-1170	Woodchips/ sewage sludge	
6	A12	9	1170-1200	Woodchips	
6	A13		1170-1200	Manure	
7		10 manure	1200-1420	Manure	
7	A14	11 hay	1200-1420	Hay	

4.4 PHYSCIAL AND CHEMICAL CHARACTERISATION OF LIQUID SAMPLES

4.4.1 *In situ* determinations – Electrical conductivity, total dissolved solids, salinity, dissolved oxygen, pH and redox potential

Figure 4.1 illustrates the electrical conductivity profile over the depth of SSRU1. Electrical conductivity was high at the influent of SSRU1, decreasing at a depth of 750 mm where after it stayed constant.

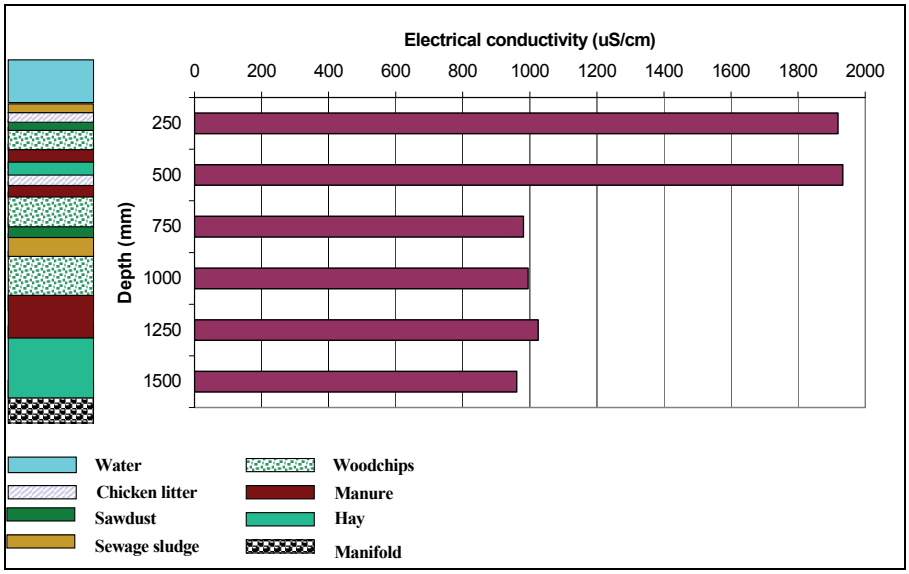


Figure 4.1: *In situ* electrical conductivity determinations over the depth of SSRU1.

The depth profile for total dissolved solids (TDS) followed the same trend as found for electrical conductivity with high TDS values in the top 500 mm of SSRU1, decreasing at a depth of 750 mm where after TDS values remain similar (Figure 4.1 and Figure 4.2).

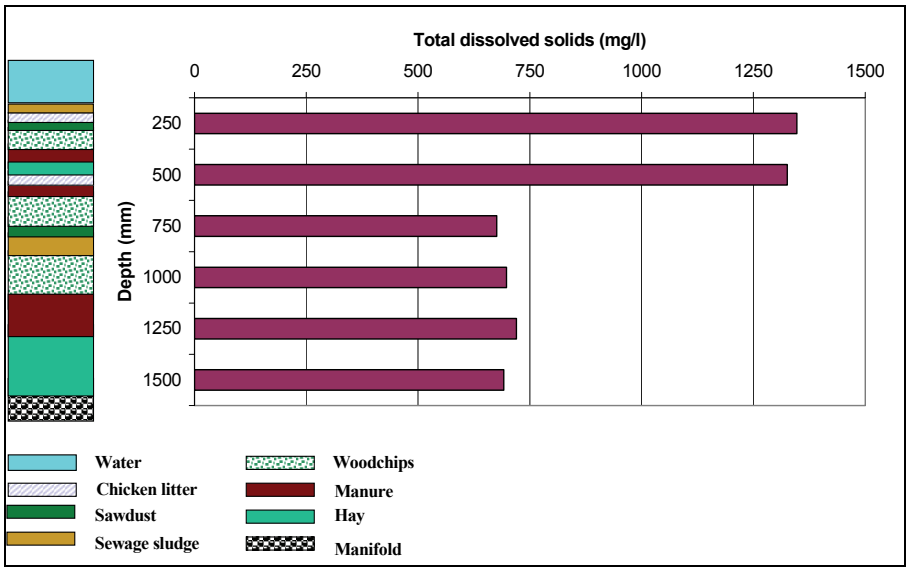


Figure 4.2: In situ total dissolved solid determinations over the depth of SSRU1.

Not surprisingly, the same depth profile was observed for salinity over the depth of SSRU1 as found for electrical conductivity and TDS (Figures 4.1, 4.2 and 4.3).

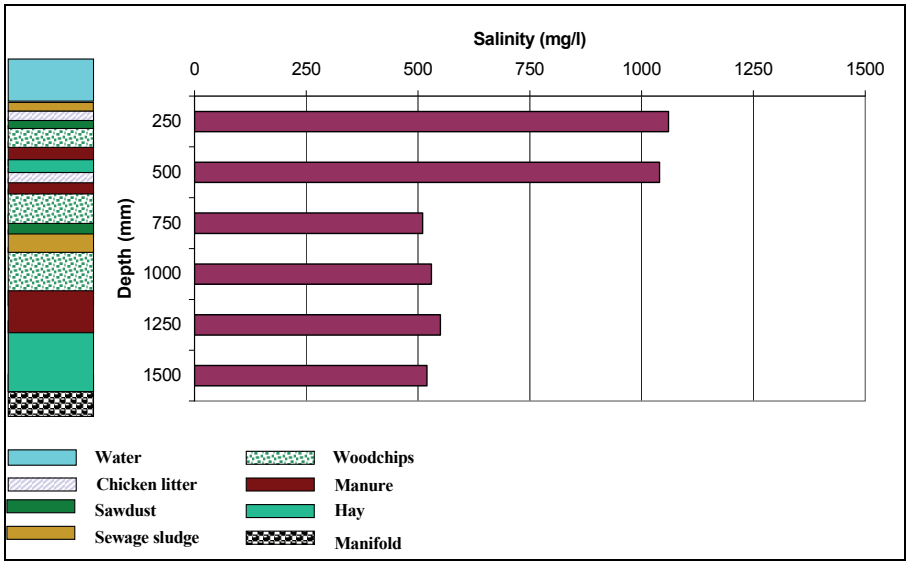


Figure 4.3: *In situ* salinity determinations over the depth of SSRU1.

In 1998, the University of Pretoria performed a study to physically, chemically and microbiologically characterise some of the SSRUs at Vryheid Coronation Colliery (VCC). It was found that the most important parameter preventing sulfate reduction, in all the units, was the prevailing oxic conditions in the bulk of the units (Cloete, 1998). The presence of oxygen in the units indicated the lack of significant microbiological activity, since the latter would have consumed the oxygen during the biodegradation of organic matter. From this it was concluded that the biodegradation of the carbon sources in the systems was the rate limiting step. Although the dissolved oxygen concentrations for the current study were in line with the results obtained in 1998, negative redox potentials were measured in SSRU1 decreasing over the depth of SSRU1 (Figure 4.5). It is believed that there must have been an error with the oxygen determinations as the redox potential values are in line with the sulfate reduction observed as well as the alkalinity produced (Figures 4.7 and 4.9).

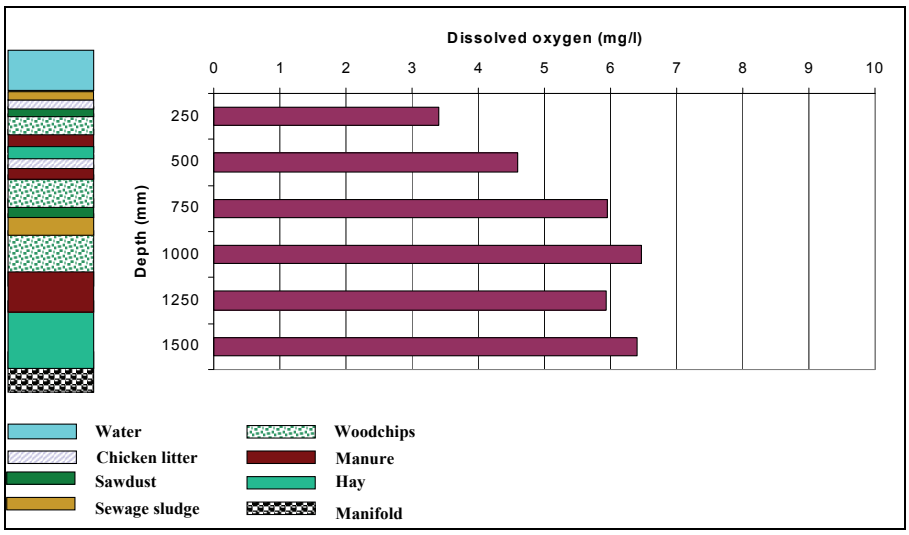


Figure 4.4: In situ dissolved oxygen determinations over the depth of SSRU1.

Redox potentials ranged between –105 and –193 mV over the depth of SSRU1 (Figure 4.5). These low redox potentials suggest that redox potential is not a limiting factor for the occurrence of sulfate reduction.

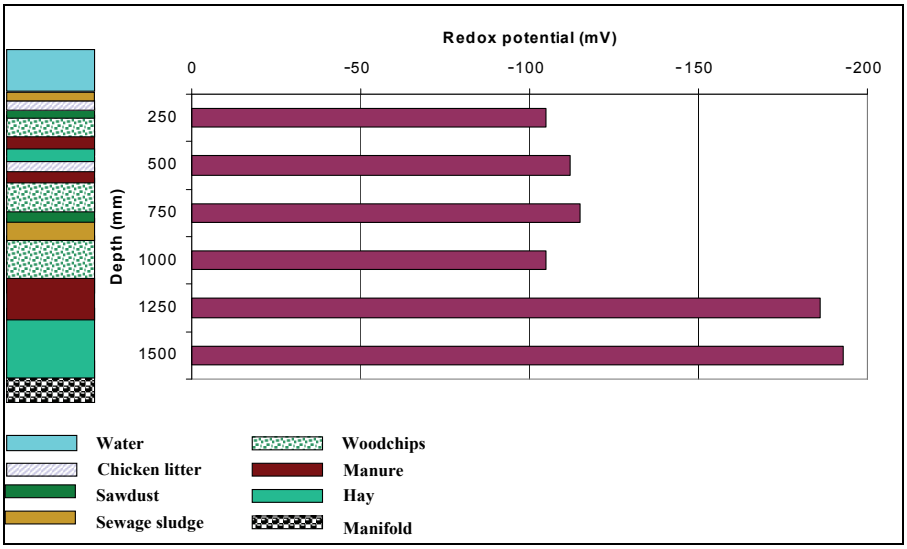


Figure 4.5: In situ redox potential determinations over the depth of SSRU1.

pH fluctuated between 5.33 and 5.71 over the depth of SSRU1 (Figure 4.8). If high rates of sulfate reduction occurred within SSRU1, it would be expected that the pH would increase deeper down SSRU1 as more alkalinity is produced. This was however not the case (Figure 4.8).

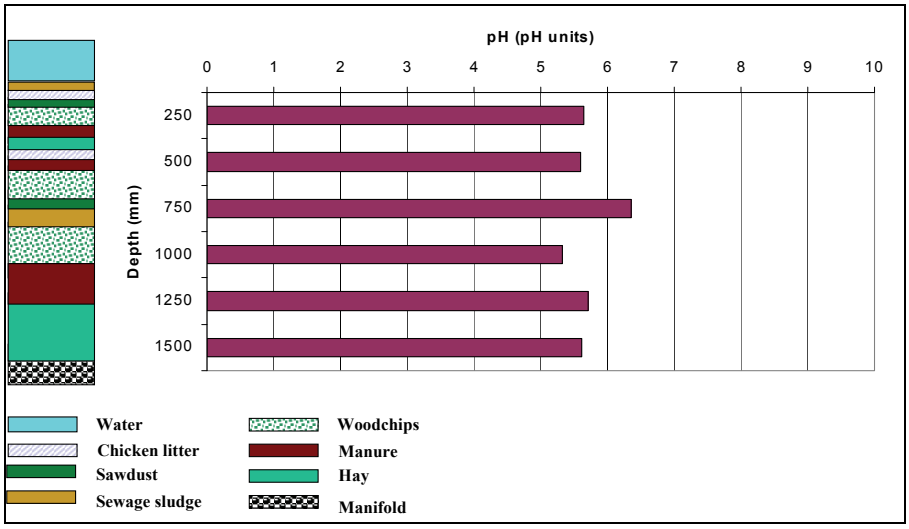


Figure 4.6: *In situ* pH determinations over the depth of SSRU1.

4.4.2 Sulfate concentration, sulfide concentration and alkalinity

Sulfate concentrations decreased over the depth of SSRU1 coinciding with an increase in sulfide concentrations (Figure 4.7 and Figure 4.8). Alkalinity as mg/ℓ $CaCO_3$ did not coincide with sulfate reduction and peaked between a depth of 500 mm and 1000 mm (Figure 4.9).

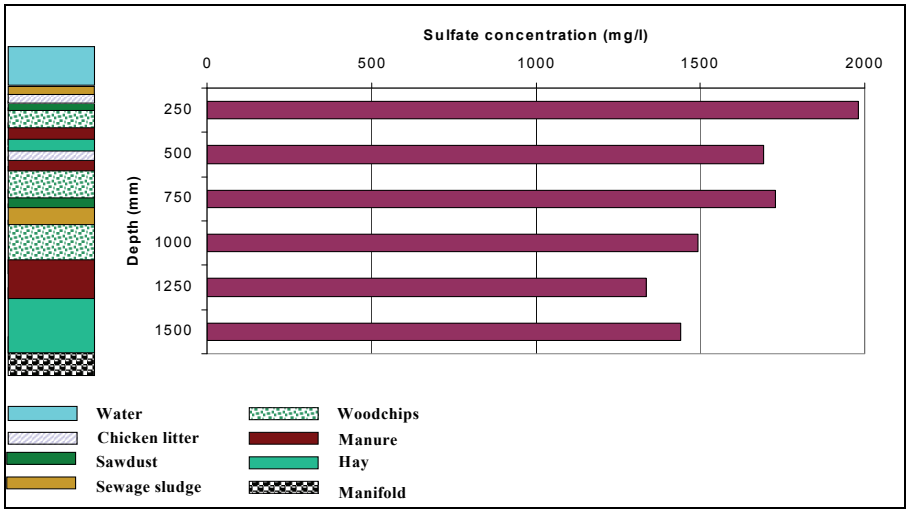


Figure 4.7: Sulfate concentrations over the depth of SSRU1.

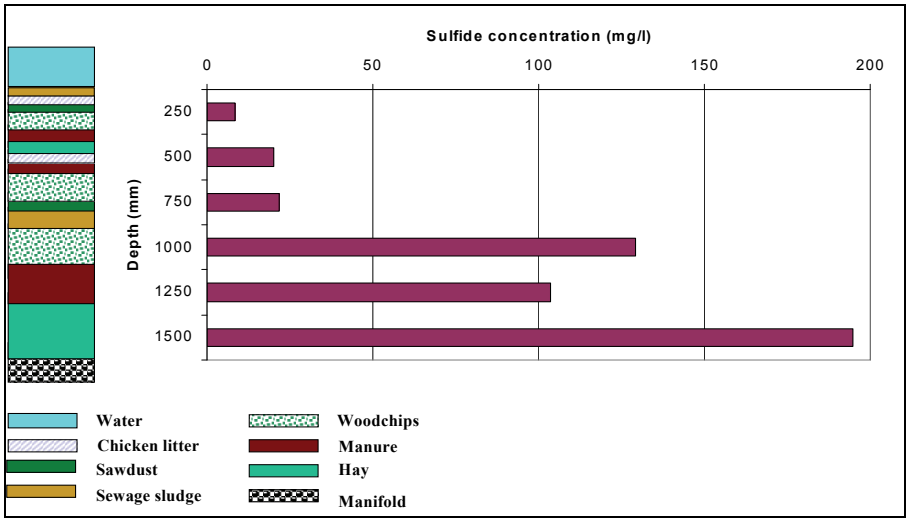


Figure 4.8: Sulfide concentrations over the depth of SSRU1.

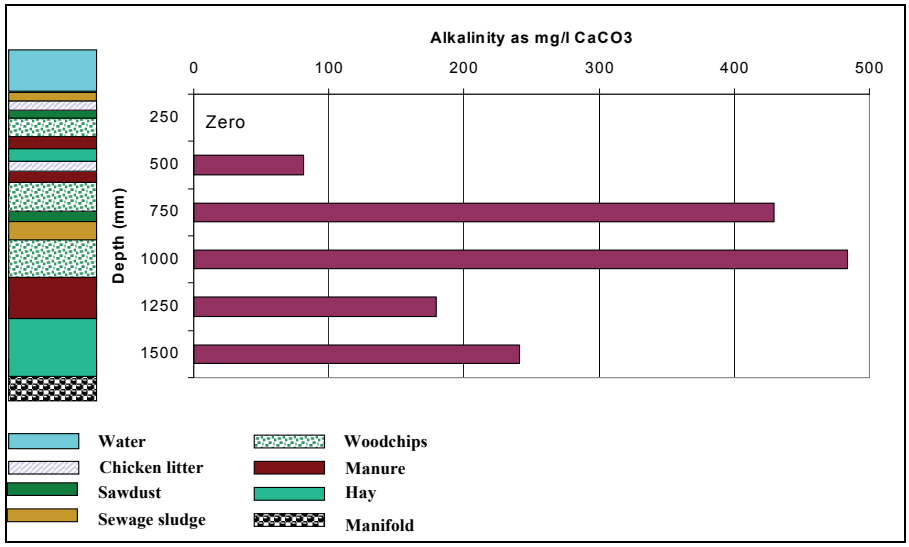


Figure 4.9: Alkalinity as mg/ℓ $CaCO_3$ over the depth of SSRU1.

4.4.3 COD concentration

High COD concentrations were observed where the manure layers were packed (Figure 4.10). Low COD concentrations were observed where woodchips and hay were packed (Figure 4.10). COD gives an indication of the total carbon available and includes the bio-available fraction of carbon available to drive biological processes.

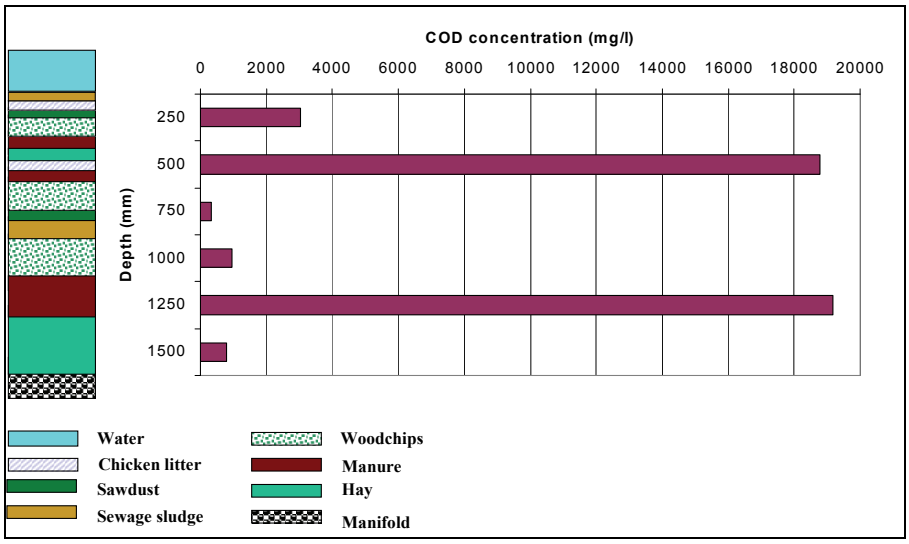


Figure 4.10: COD concentrations over the depth of SSRU1.

4.4.4 Metal concentrations

Figures 4.11-4.13 illustrate the depth profiles of the various metals analysed in SSRU1. Low aluminium, cobalt and lead concentrations were observed over the depth of SSRU1 (Figure 4.11). Metal concentrations (iron, manganese, potassium, sodium, calcium and magnesium) fluctuated over the depth of SSRU1 and no particular trends can be distinguished (Figure 4.12 and Figure 4.13).

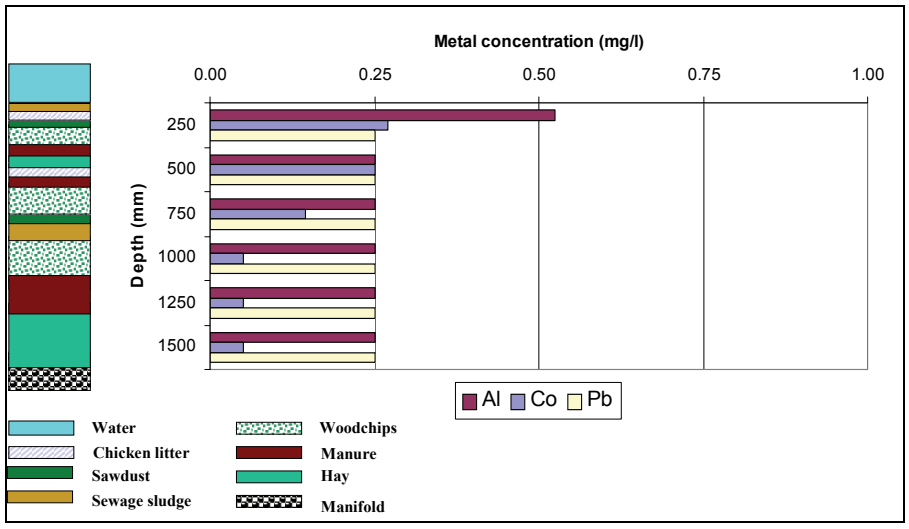


Figure 4.11: Aluminium-, cobalt- and lead concentrations over the depth of SSRU1.

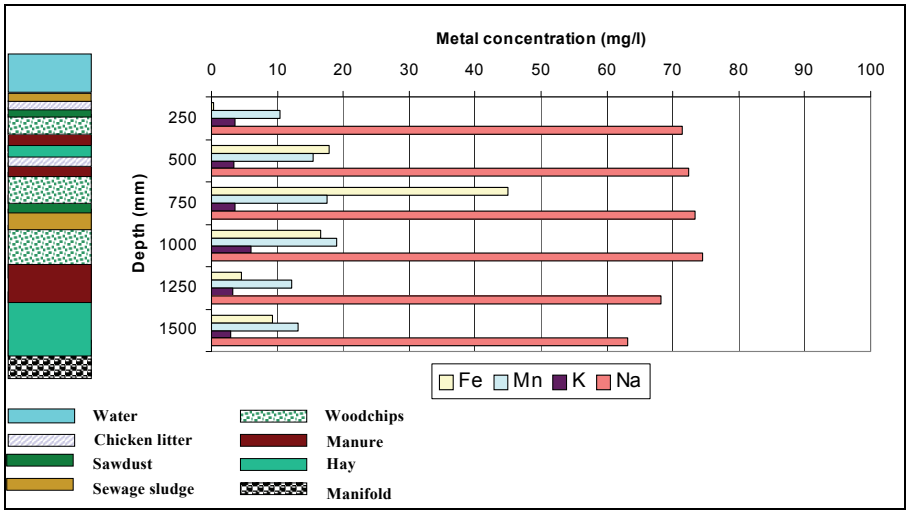


Figure 4.12: Iron-, manganese-, potassium- and sodium concentrations over the depth of SSRU1.

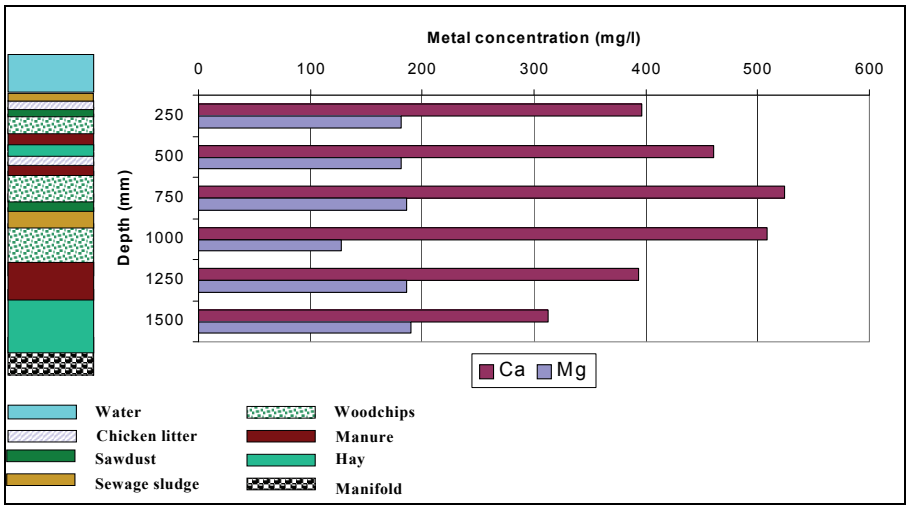


Figure 4.13: Calcium- and magnesium concentrations over the depth of SSRU1.

4.4.5 Starch, water soluble carbohydrates (WSC) and total non-structural carbohydrates (TNC)

Very low TNC values were found over the depth of SSRU1 and therefore the ARC did not perform starch and WSC determinations on the samples (Table 4.2). The low TNC values observed can either indicate that all of the available TNC is utilised microbiologically or that the lignocellulose degradation is limited.

Table 4.2: Total non-structural carbohydrates (TNC) over the depth of SSRU1.

Depth from top (mm)	Total non-structural carbohydrates (TNC) (%)
0-250	0.05
251-500	0.15
501-750	0.00
751-1000	0.00
1001-1250	0.29
1251-1500	0.01

4.4.6 Aromatic compounds

The standard curve produced to determine the aromatic compounds using ferulic acid is given in Figure 4.14. Figure 4.15 illustrates the concentrations of aromatic compounds over the depth of SSRU1. The results show that there are more lignin derived aromatic compounds present in the lower layers of the reactor (4-6) than in the upper layers of the reactor (1-3) (Figures 4.14 and 4.15). This does not necessarily suggest a higher level of microbial activity in the lower layers, or that the microbes present there are attacking the lignocellulose complex. The components observed here could have been carried down from the upper layer. Nevertheless, the observation of aromatic compounds is a clear indication of lignocellulose breakdown.

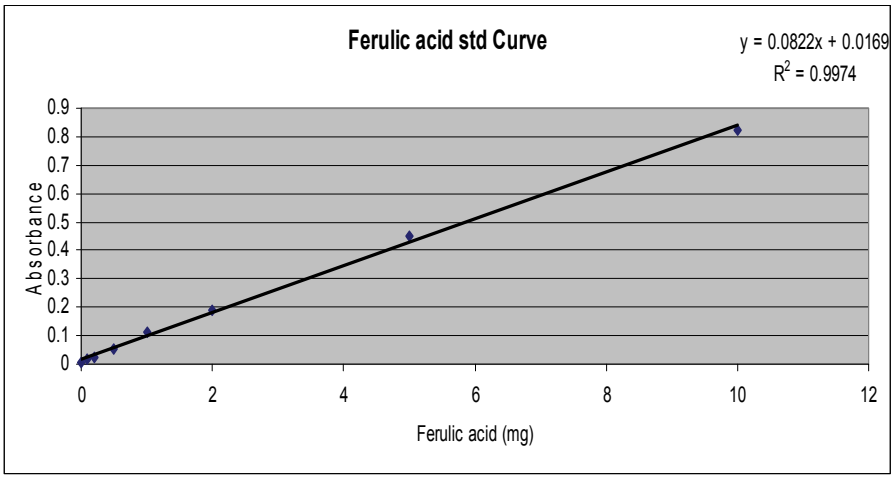


Figure 4.14: A Standard curve using Ferulic acid.

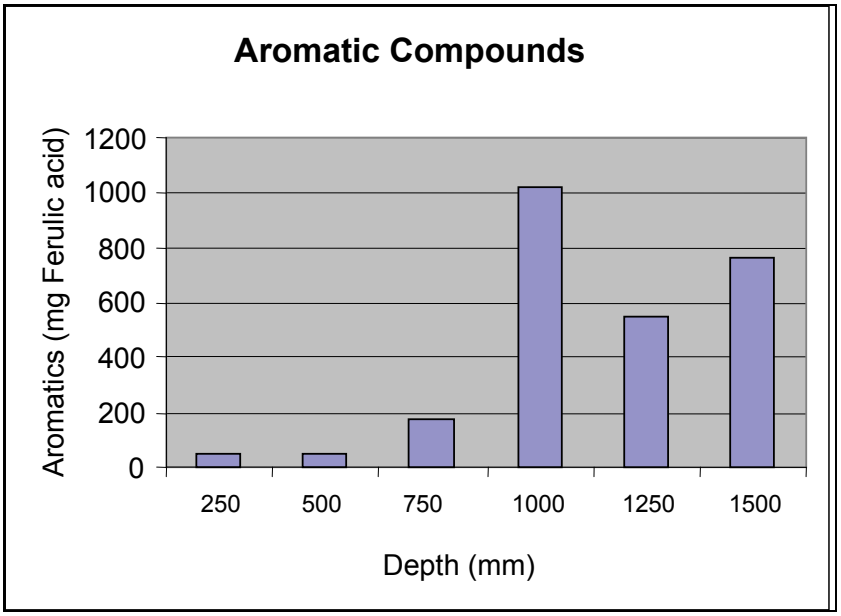


Figure 4.15: Aromatic compounds present in the liquid samples.

4.4.7 Total organic carbon

The results for total organic carbon are shown in Figure 4.16. The results show that there is more TOC present in the lower liquid layers than in the upper layers (Figure 4.16) which corresponds to the observation made above. This could be due to the bacteria in either the upper or the lower layers releasing TOC into the liquid fraction, as they break down the lignocellulose complex.

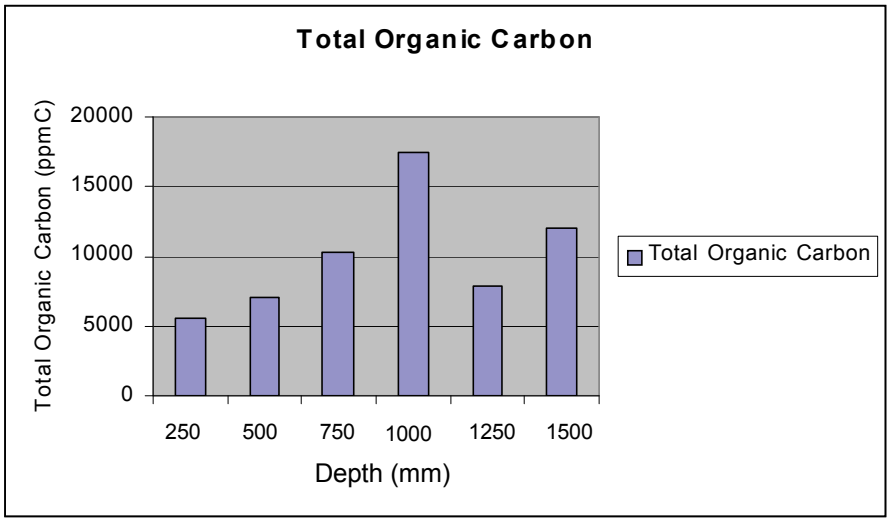


Figure 4.16: TOC of the different liquid fractions.

4.4.8 Volatile fatty acids

Figure 4.17 illustrates VFA concentrations over the depth of SSRU1. The graph indicated that the Top and Bottom layers had low levels of VFA and that the Middle layers had higher levels. This could indicate that bacteria growing in the Top and Bottom layers are utilizing the VFA in solution more actively than in the Middle.

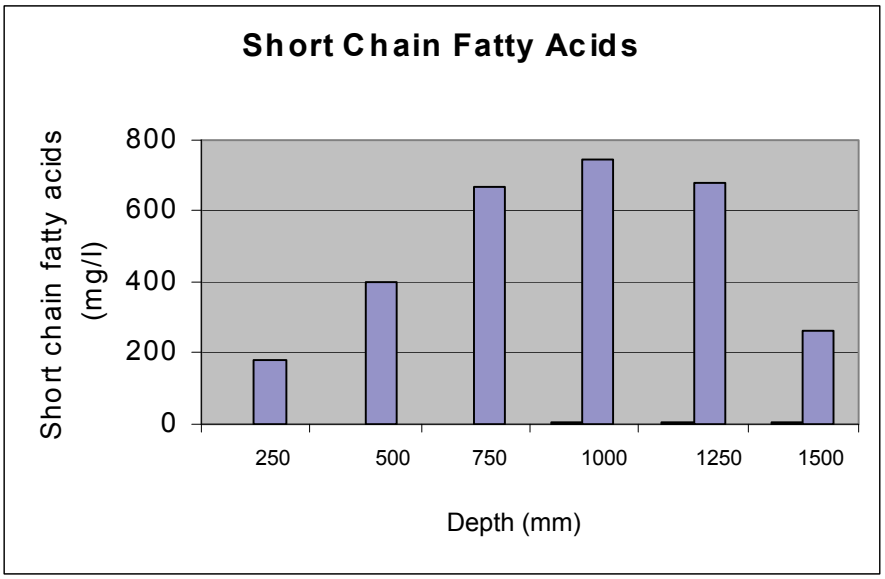


Figure 4.17: Short chain fatty acid results of the liquid samples.

4.4.9 Reducing sugars

The reducing sugar results are seen in Figure 4.18. The results indicate that all the reducing sugars near the bottom of the reactor have been exhausted or converted to VFA or that cellulose breakdown is more active in the upper layer. The highest levels of reducing sugars are near the surface. When the reducing sugar results (Figure 4.18) are compared to the VFA results (Figure 4.17) it appears that the reducing sugars may have been broken down into VFA at the bottom of the reactor, or that degradation is occurring more rapidly in the Top layer. These findings concur to some extent with the extraction and SEM studies reported in Section 4.3.7.

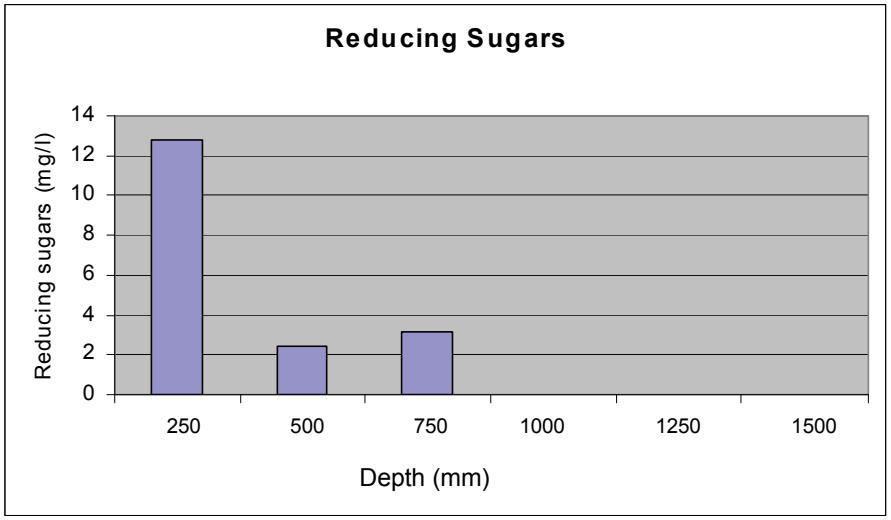


Figure 4.18: Reducing sugar results for the liquid samples.

4.5 PHYSICAL AND CHEMICAL CHARACTERISATION OF SOLID SAMPLES

4.5.1 Protein content, fat content and *in vitro* digestibility

Low protein content (%) was observed for the different carbon sources, ranging between 0.62% and 7.78% (Figure 4.19). The highest percentage protein content was found in the sewage sludge/chicken litter mixture (7.78%) and in the manure/hay mixture (6.7%). The protein content for the wood chips (not extracted) was higher than the washed wood chips (not extracted) (Figure 4.19). This could be expected as carbon with smaller particle sizes *e.g.* manure migrated in-between the woodchips leading to the higher percentage protein content. Low percentage fat content was measured in the different carbon sources (Figure 4.19). Fresh pine chips had the highest percentage fat content (2.41%).

Manure was the only carbon source having tested positive for *in vitro* digestibility (18.54%) (Figure 4.19). The higher *in vitro* digestibility associated with the manure could be expected as manure is one of the carbon sources with the lowest lignin content and the highest biodegradability. The *in vitro* digestibility associated with the woodchips which were not extracted with water, could be attributed to manure being present with the woodchips. Although the fresh pine chips had some degree of *in vitro* digestibility, it was low (3.88%).

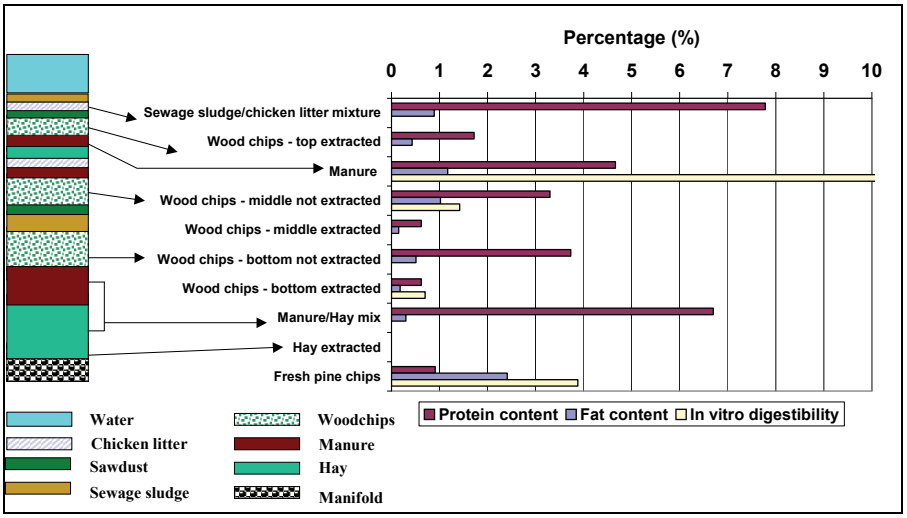


Figure 4.19: Protein content, fat content and *in vitro* digestibility

4.5.2 Lignin, hemicellulose and cellulose

As could be expected, the highest percentage lignin, cellulose and hemi-cellulose content were found for the woodchips at the different depths (Figure 4.20). The percentages lignin, cellulose and hemi-cellulose of the fresh pine chips were lower than for the woodchips at the different levels (Figure 4.20). This could be attributed to the fact that the fresh pine chips have not been subjected to any degradation processes and that not only the more recalcitrant carbon portion is left as would be the case with the other woodchips samples.

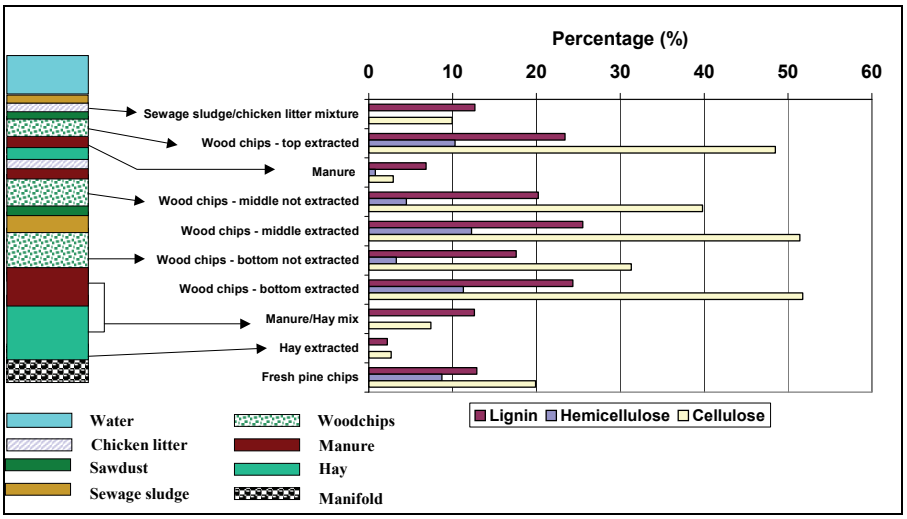


Figure 4.20: Lignin, hemicellulose and cellulose.

4.5.3 Moisture content, ash content and crude fibre content

Figure 4.21 summarises the percentage moisture content, ash content and crude fibre content of the different carbon sources investigated. The sewage sludge/chicken litter mixture and the manure/hay mixture had the highest ash content (Figure 4.21).

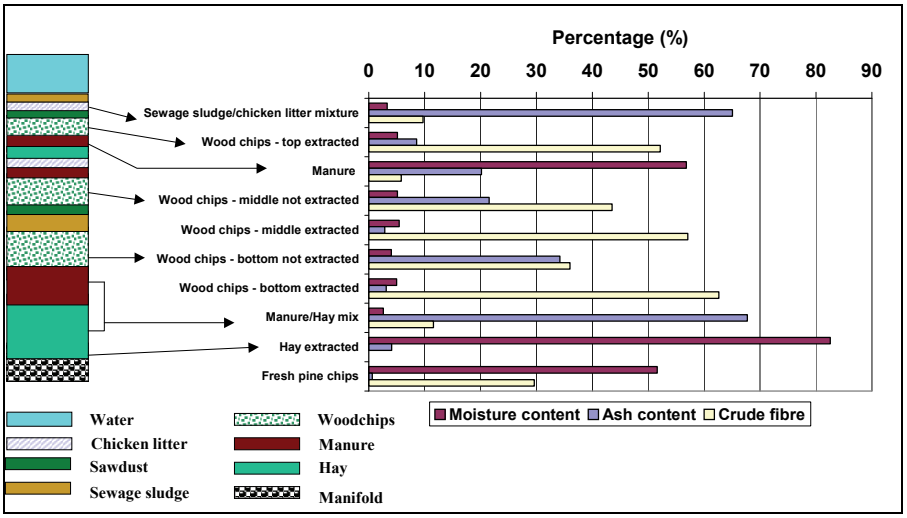


Figure 4.21: Moisture content, ash content and crude fibre.

4.5.4 Aqua regia digest

Figures 4.22-4.25 summarises the metal content found for the different carbon sources after aqua regia digest. A phenomena which has been seen previously, namely that the original carbon sources contain metals which could potentially leach from the carbon and contribute to metal production in the system was once again seen with the results obtained for fresh pine chips. The fresh pine chips contain various metals which could potentially leach from the carbon (Figure 4.22-4.25). The high metal content found for the other carbon sources indicate that metal precipitation occurred within the system through the formation of metal sulfides (Figure 4.22-4.25).

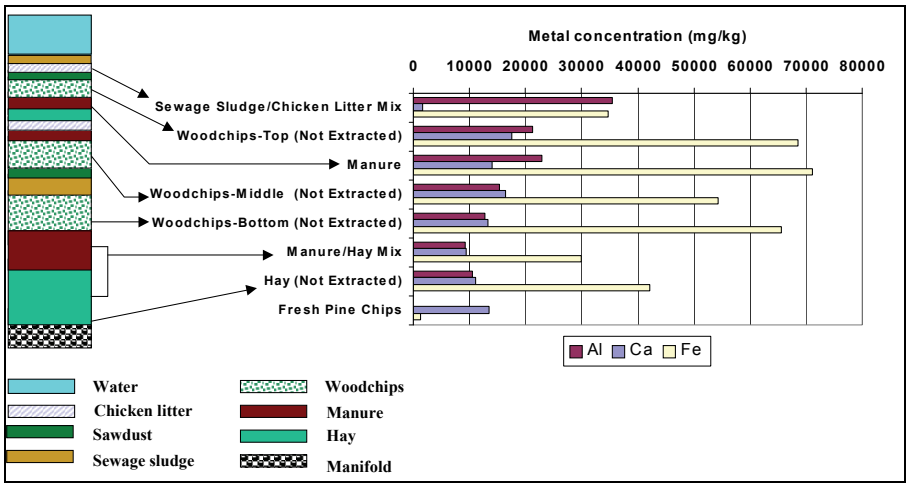


Figure 4.22: Aluminium-, calcium- and iron concentrations after aqua regia digest of the different carbon sources.

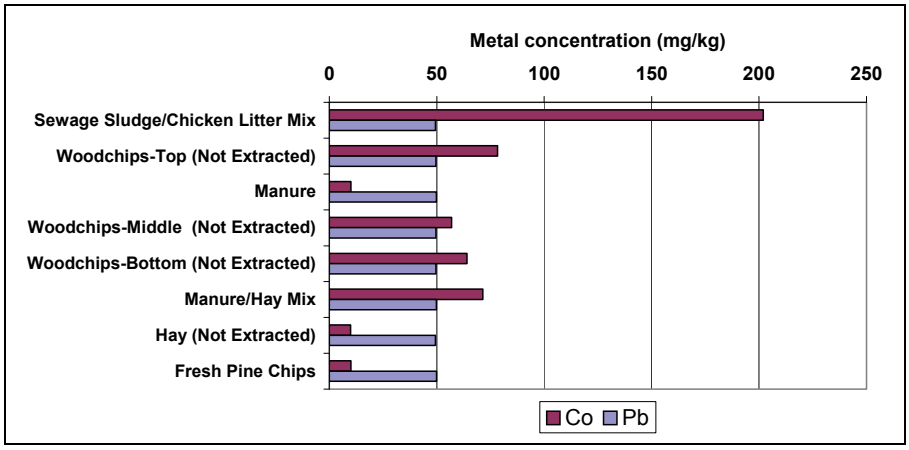


Figure 4.23: Cobalt- and lead concentrations after aqua regia digest of the different carbon sources.

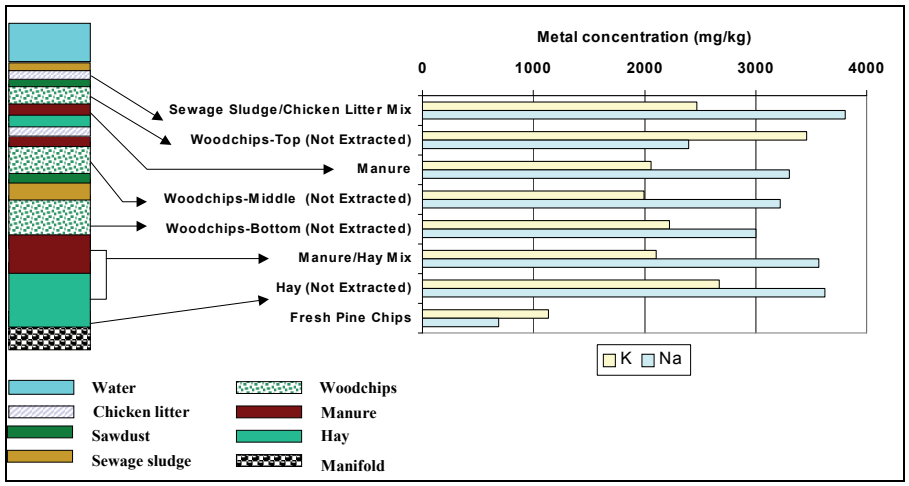


Figure 4.24: Potassium- and sodium concentrations after aqua regia digest of the different carbon sources.

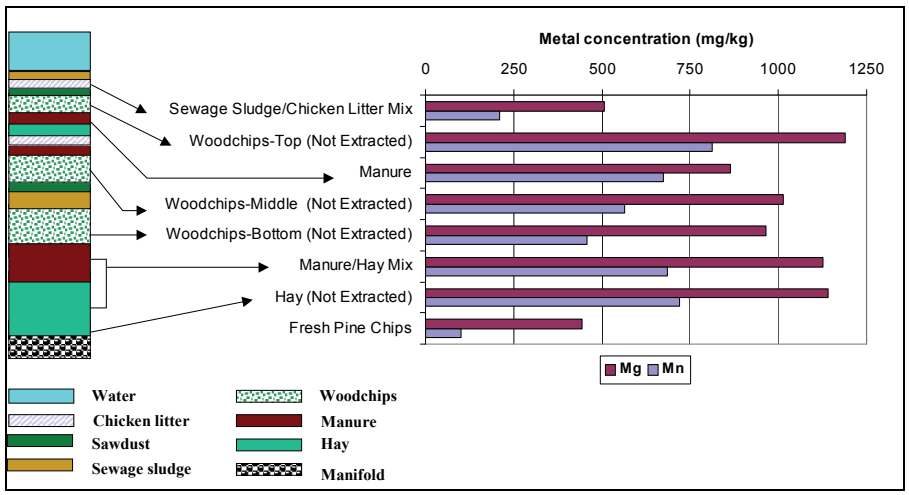


Figure 4.25: Magnesium- and manganese concentrations after aqua regia digest of the different carbon sources.

4.5.5 Water extraction

Figures 4.26-4.28 illustrates the metal concentrations found for the different carbon sources after extraction with water. The manure/hay mix had the highest metal content after extraction for all of the metals analysed (Figures 4.26-4.28).

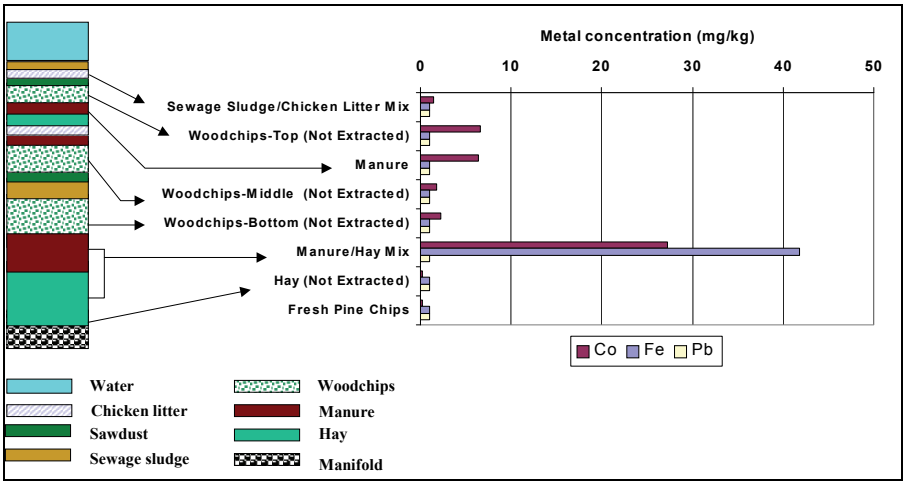


Figure 4.26: Cobalt-, iron- and lead concentrations after water extraction of the different carbon sources.

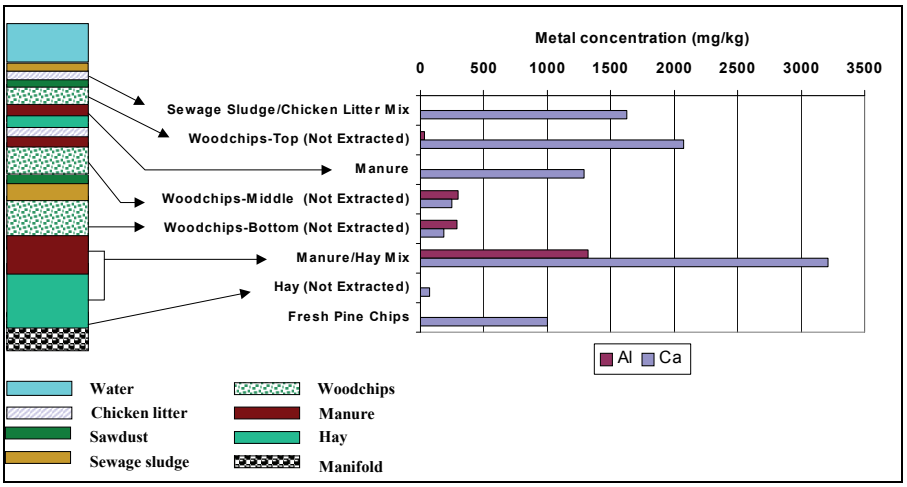


Figure 4.27: Aluminium- and calcium concentrations after water extraction of the different carbon sources.

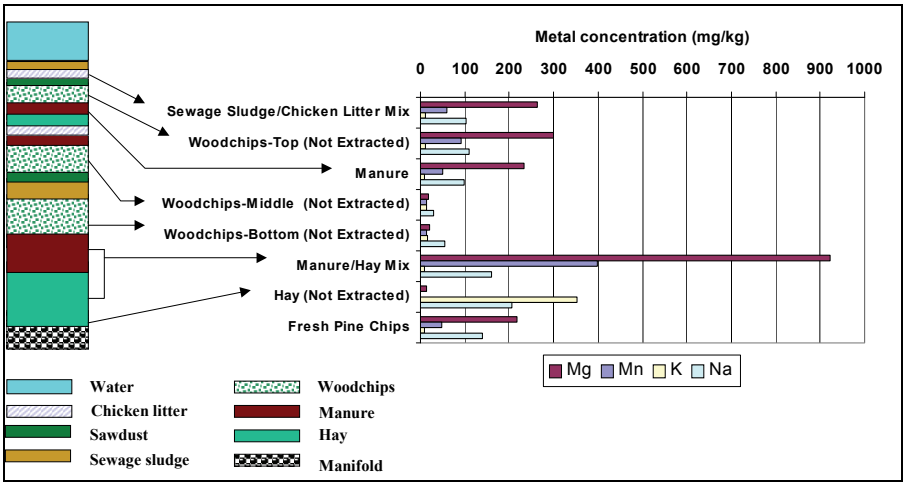


Figure 4.28: Magnesium-, manganese-, potassium- and sodium concentrations after water extraction of the different carbon sources.

4.5.6 Analysis of soluble, pectin and lignocellulose fractions

Figure 4.29 shows the percentage of soluble extractives of the different wood samples and Figure 4.30 shows the percentage pectin of the different wood samples.

The residual percentage of the lignocellulose complex remaining after the removal of the soluble and pectin fractions is shown in Figure 4.31, and Figure 4.32 presents a summary of the woodchip extraction study. It is evident from these results that the fresh wood sample does not provide a satisfactory control, and that it would have been useful to have had an unutilised sample from the original packing material for comparative purposes.

The results showed that the middle and lower woodchip samples contained the greatest percentage of soluble wood extractives (Figure 4.29). This suggests that the wood in the upper part of the reactor has been more successfully opened up by microbial and/or chemical attack, allowing for the release of the soluble wood extractives. However, the percentage of pectin was slightly higher in the top compared to the middle and lower fractions (Figure 4.30). This suggests that while solubles remained present in the wood in the lower sections of the reactor, that the pectin was to some extent also being mobilised in this fraction.

Notwithstanding these results it is evident that little change in the lignocellulose fraction has occurred through the years over which the reactor has been operated. This is further confirmed in the scanning electron microscopy study (Section 4.3.7).

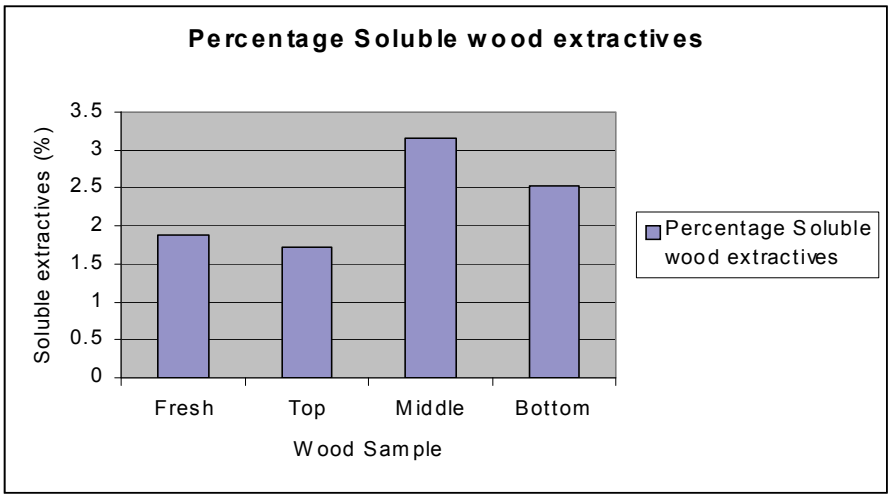


Figure 4.29: Percentage (dry weight) soluble wood extractives of the different wood samples.

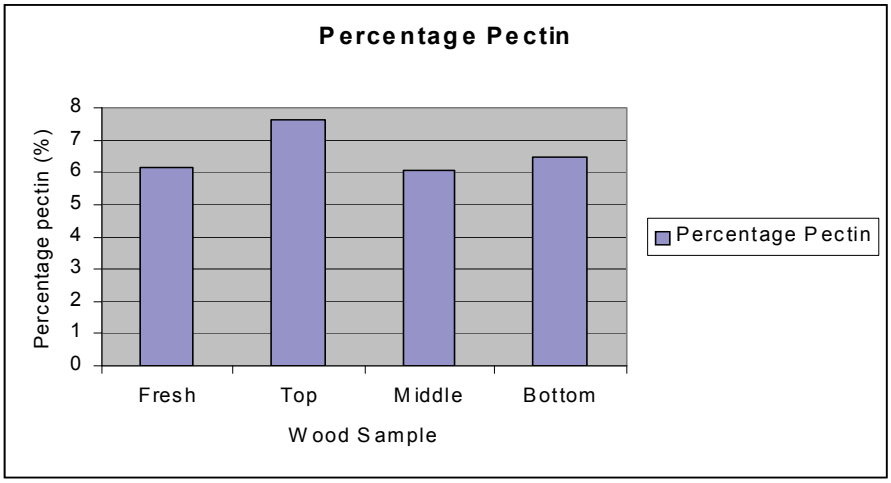


Figure 4.30: Percentage (dry weight) pectin in the different wood samples.

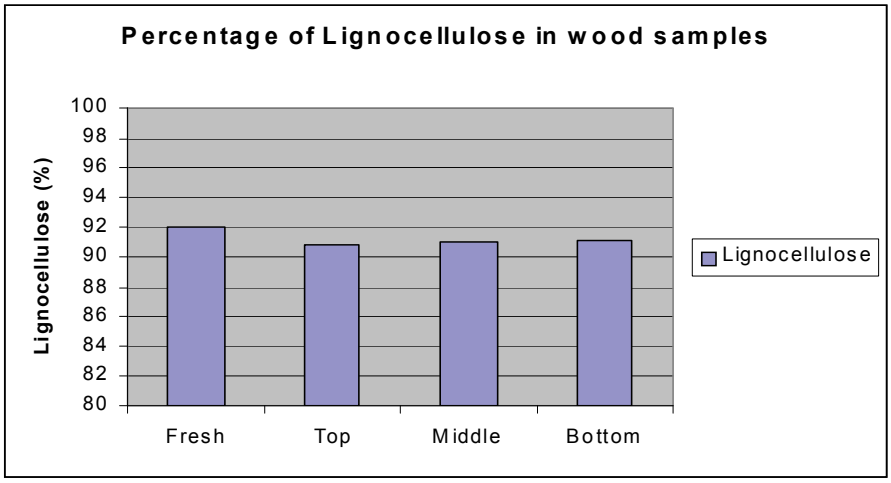


Figure 4.31: Lignocellulose complex fraction remaining in the control and samples.

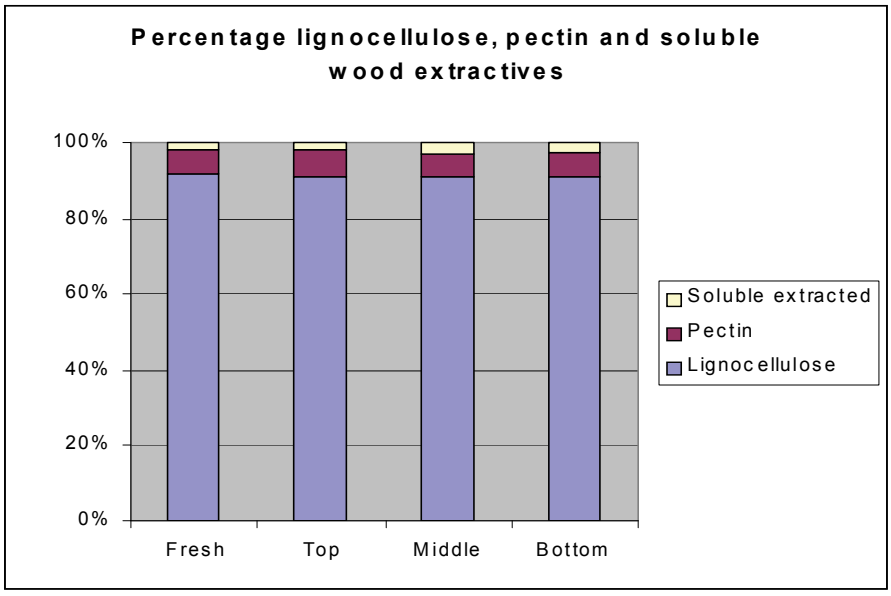


Figure 4.32: Comparison of the changes in the various fractions in the woodchip control and samples.

4.5.7 Scanning electron microscopy of woodchip fractions

Figures 4.33 to 4.39 show the structure of the woodchips at the 3 packing levels and also the control sample. The SEM results show that the structure of the fresh wood control (Figure 4.33) remains largely intact, no structural pores are visible. In Figure 4.34 showing woodchips from the Top layer, the structural pores are clearly visible, indicating partial degradation of the surface layers. Figure 4.35 shows that wood from the Top layer, has a clearly defined structure. Woodchips from the Middle and Bottom layers (Figure 4.36, 4.37, 4.38 and 4.39) seem to be largely intact as they closely resemble the fresh wood, in that the pores are not clearly visible. This observation accords to some extent with the observation that the soluble extractable fraction was higher at these two layers suggesting reduced attack at the lower locations in the reactor.

Figure 4.33: SEM of the fresh wood control (350X).

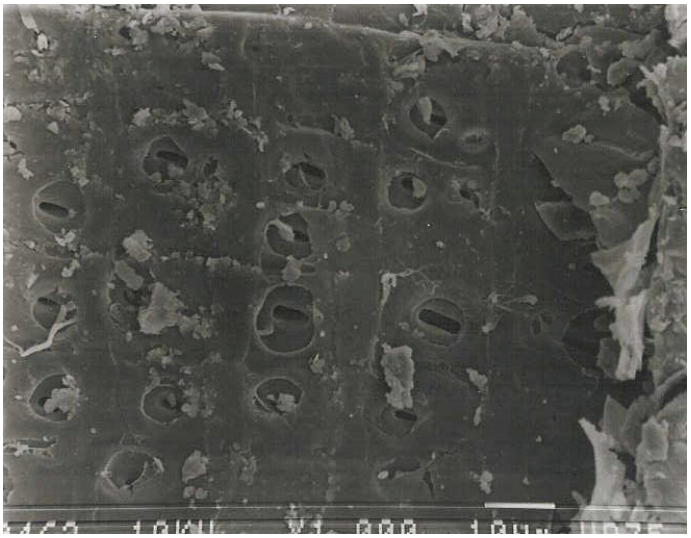


Figure 4.34: SEM of the woodchip sample from the Top layer (1000X).

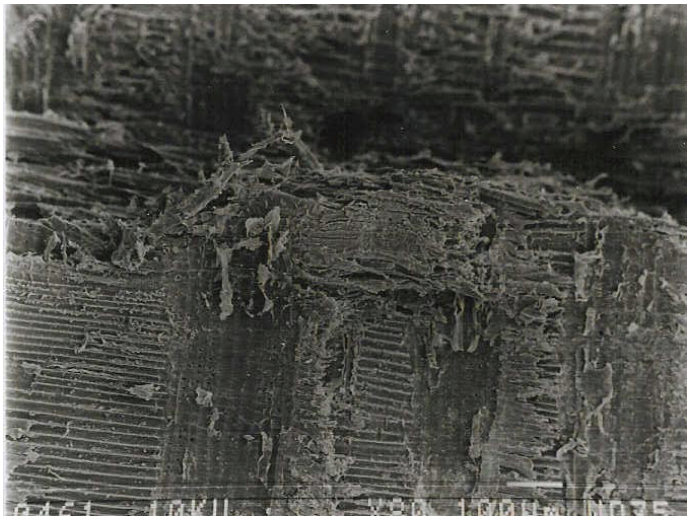


Figure 4.35: SEM of the woodchip sample from the Top layer (80X).

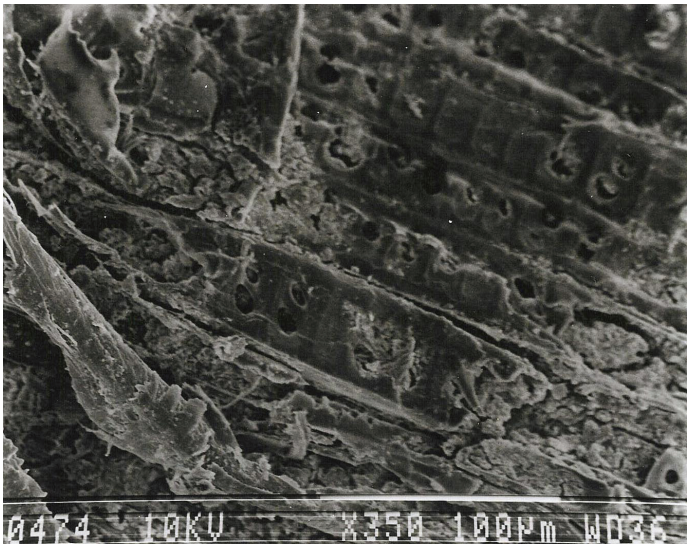


Figure 4.36: SEM of the woodchip sample from the Middle layer (350X).

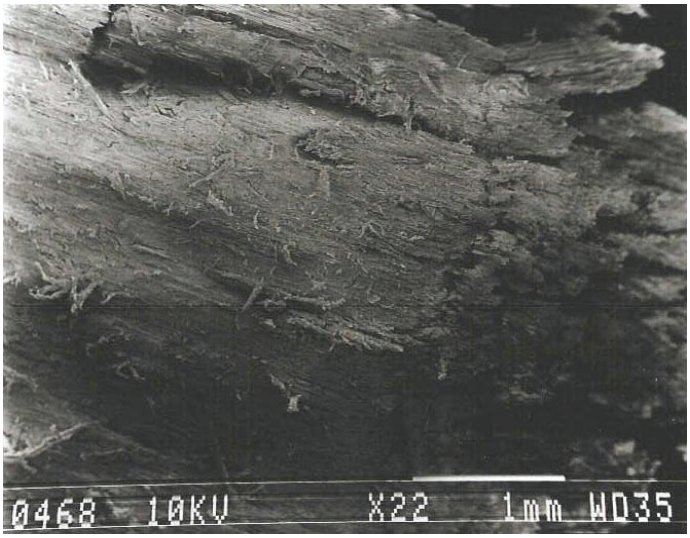


Figure 4.37: SEM of the woodchip sample from the Middle layer (22X).

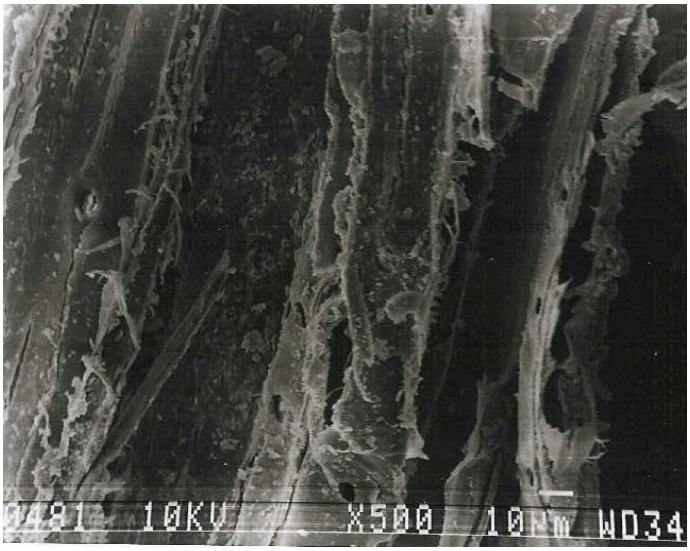


Figure 4.38: SEM of the woodchip sample from the Bottom layer (500X).

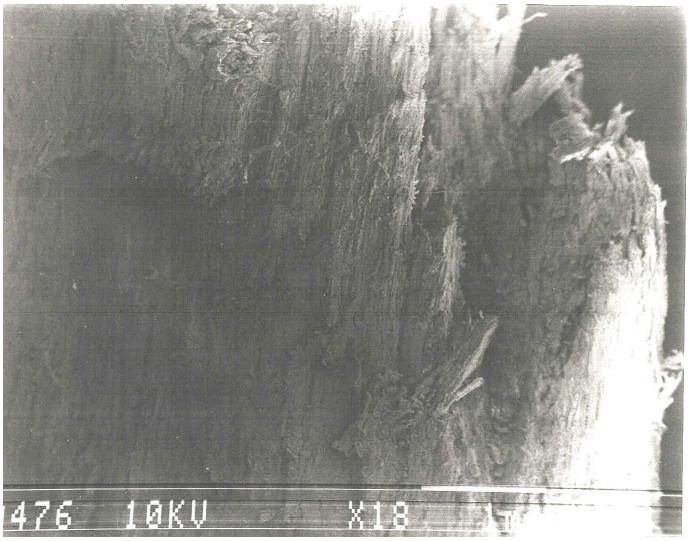


Figure 4.39: SEM of the woodchip layer from the Bottom layer (18X).

4.6 MOLECULAR STUDIES

Figures 4.40-4.42 show the agarose gels from which the results were derived for the DNA extraction, PCR and Eco RI digest which formed part of the phylogenetic study.

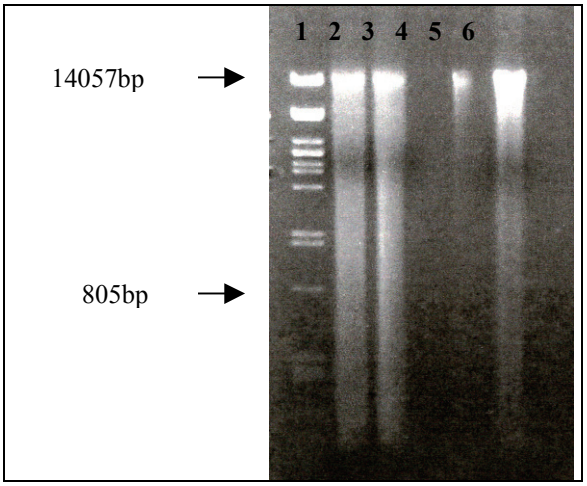


Figure 4.40: Agarose gel showing the results of the DNA extraction.

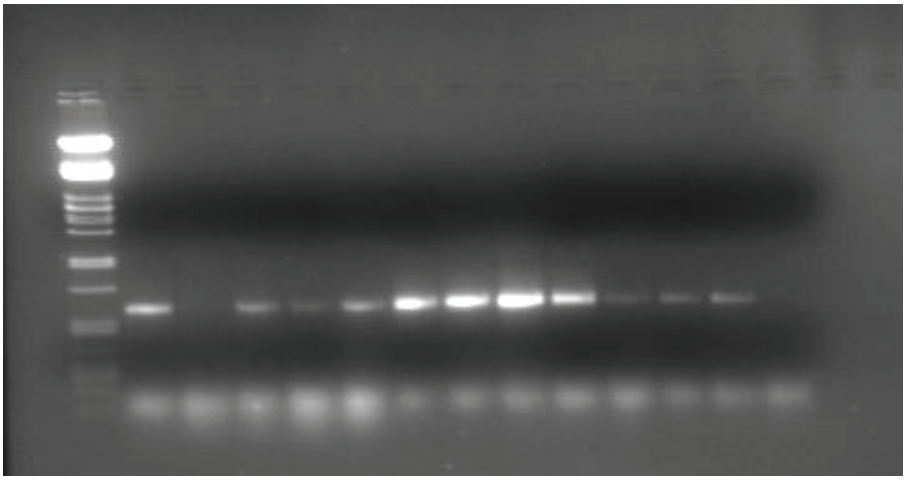


Figure 4.41: Agarose gel showing the PCR results.

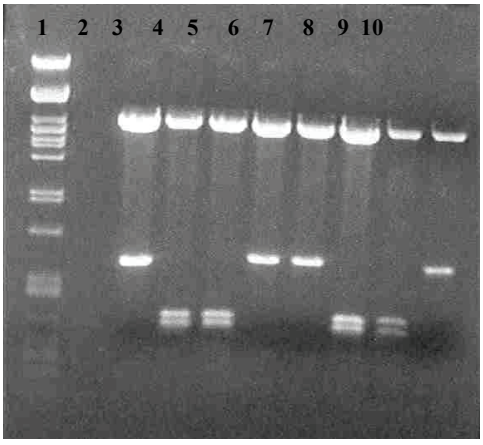


Figure 4.42: Agarose gel results showing the results of the Eco RI digest.

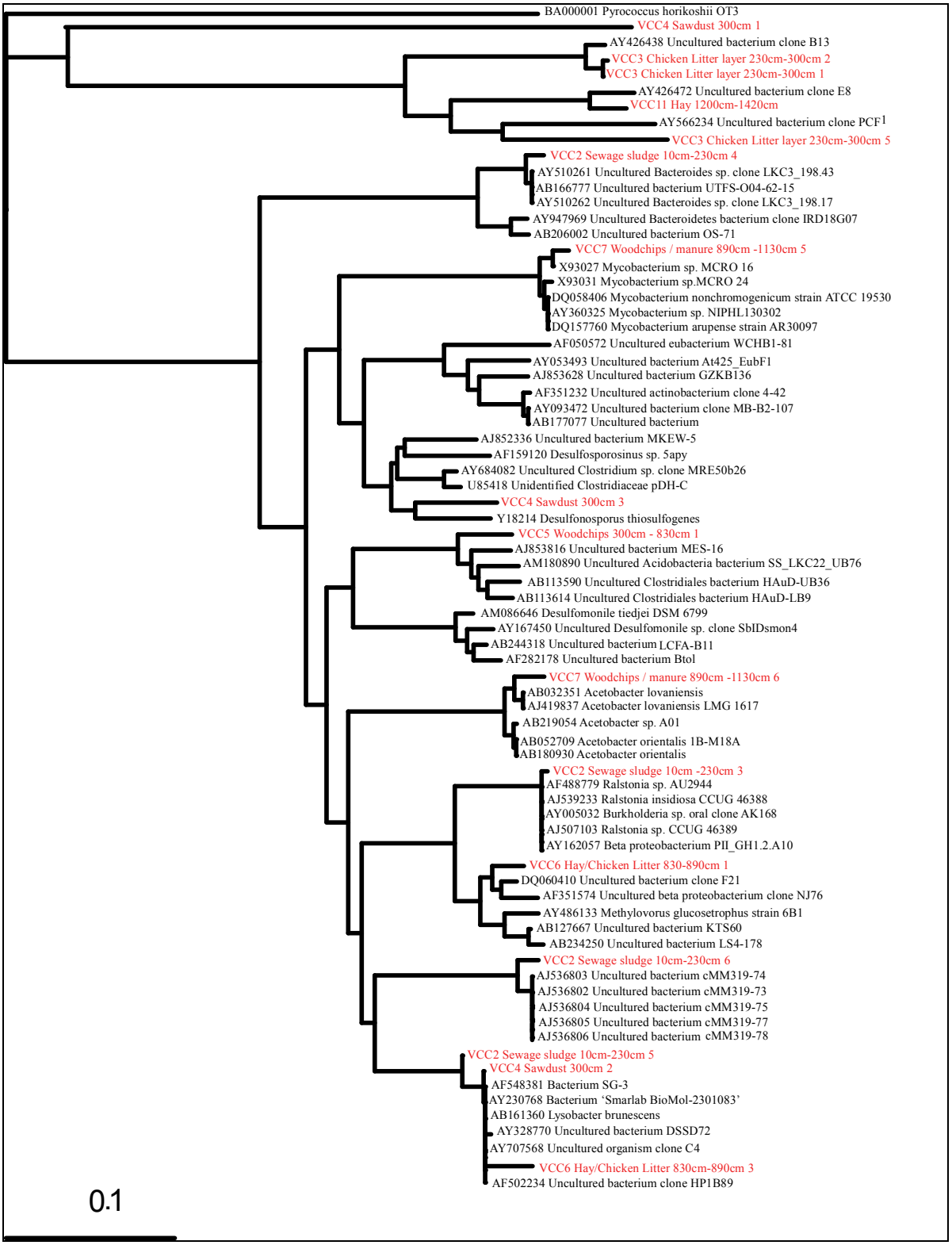


Figure 4.43: Phylogenetic Tree showing the results of the VCC molecular analysis

The analysis of the results from the molecular phylogenetic study is described in the following section. The results of the phylogenetic study are presented in two forms, the phylogenetic tree and the tables showing the closest BLAST matches, the most important being the phylogenetic tree (Figure 4.43). The phylogenetic tree depicts how each clone is related to each other clone and to the top five BLAST matches. Species on the same branch are very closely related to each other. The tables (5-11)

display the top 5 BLAST matches for each clone as downloaded from the NCBI website. Only species with a 95% or greater match to the clones are taken into consideration. Although there is some spread of opinion on the topic, matches between 95% and 97% are considered to imply species-level relatedness. The BLAST matches are not necessarily the clones' closest relative, and this can be seen on the phylogenetic tree (Figure 4.43). Using the phylogenetic tree and the match tables the VCC clones can be compared to other species on the BLAST website.

VCC Sample 2 (Sewage Sludge Layer 10-230 mm)

VCC2 Clones were found in the sewage sludge layer which occurred 10 mm to 230 mm from the surface of the reactor. Table 4.3 reports details of the clones selected along with their 5 closest matches on the BLAST database. Available details on physiological status of the known organisms are presented and provide an indication of structure and functional relationships in the system under investigation.

Table 4.3: Table detailing the VCC2 clones found in the sewage sludge layer 10-230 mm from the top.

VCC2 Clone 3	Taxonomy	Origin of Blast strain
AY005032 *Burkholderia sp.* oral clone AK168 16S ribosomal RNA gene	Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales; Burkholderiaceae; Burkholderia; environmental samples.	Bacterial diversity in human subgingival plaque. JOURNAL J. Bacteriol. 183 (12), 3770-3783 (2001)
AF488779 *Ralstonia sp.* AU2944 16S ribosomal RNA gene	Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales; Burkholderiaceae; Ralstonia.	Classification of *Ralstonia pickettii*-like isolates from the environment and clinical samples as *Ralstonia insidiosa sp.* nov JOURNAL Int. J. Syst. Evol. Microbiol. 53 (4), 1075-1080 (2003)
AJ539233 *Ralstonia insidiosa* partial 16S rRNA gene, strain CCUG 46388. ACCESSION AJ539233	Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales; Burkholderiaceae; Ralstonia.	Wautersia gen. nov., a novel genus accommodating the phylogenetic lineage including *Ralstonia eutropha* and related species, and proposal of *Ralstonia* [Pseudomonas] *syzygii* (Roberts et al., 1990) comb. nov JOURNAL Int. J. Syst. Evol. Microbiol. 54 (Pt 2), 317-327 (2004)
AJ507103 *Ralstonia sp.* CCUG 46389 partial 16S rRNA gene, type strain CCUG 46389.	Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales; Burkholderiaceae; Ralstonia.	Wautersia gen. nov., a novel genus accommodating the phylogenetic lineage including *Ralstonia eutropha* and related species, and proposal of *Ralstonia* [Pseudomonas] *syzygii* (Roberts et al., 1990) comb. nov JOURNAL Int. J. Syst. Evol. Microbiol. 54 (Pt 2), 317-327 (2004)
AY162057 Beta proteobacterium PII_GH1.2.A10 small subunit ribosomal RNA gene, partial sequence.	Bacteria; Proteobacteria; Betaproteobacteria	Cultivating the uncultured JOURNAL Proc. Natl. Acad. Sci. USA 99 (24), 15681-15686 (2002) PUBMED 12438682
VCC2 Clone 4		
AY510262 Uncultured *Bacteroides sp.* clone	Bacteria; Bacteroidetes; Bacteroidetes (class);	Bacterial diversity and ecosystem function of filamentous microbial

LKC3_198.17 16S ribosomal RNA gene, partial sequence.	Bacteroidales; Bacteroidaceae; Bacteroides; environmental samples.	mats from aphotic (cave) sulfidic springs dominated by chemolithoautotrophic 'Epsilonproteobacteria' JOURNAL FEMS Microbiol. Ecol. 51 (1), 31-53 (2004)
AY510261 Uncultured *Bacteroides sp.* clone LKC3_198.43 16S ribosomal RNA gene, partial sequence.	Bacteria; Bacteroidetes; Bacteroidetes (class); Bacteroidales; Bacteroidaceae; Bacteroides; environmental samples.	Bacterial diversity and ecosystem function of filamentous microbial mats from aphotic (cave) sulfidic springs dominated by chemolithoautotrophic 'Epsilonproteobacteria' JOURNAL FEMS Microbiol. Ecol. 51 (1), 31-53 (2004)
AB166777 Uncultured bacterium gene for 16S ribosomal RNA, partial sequence,clone:UTFS-O04-62-15.	Bacteria; environmental samples	In situ detection and identification of bacteria responsible for enhanced biological phosphorus removal in activated sludge
AB206002 Uncultured bacterium gene for 16S rRNA, partial sequence, clone:OS-71.	Bacteria; environmental samples.	Identification of Acetate- or Methanol-Utilizing Bacteria under Denitrifying Conditions by Stable-Isotope Probing
AY947969 Uncultured Bacteroidetes bacterium clone IRD18G07 16S ribosomal RNA gene, partial sequence.	Bacteria; Bacteroidetes; environmental samples.	Synchrony and seasonality in bacterioplankton communities of two temperate rivers JOURNAL Limnol. Oceanogr. 50 (6), 1718-1729 (2005)
VCC2 Clone 5		
AY230768 Bacterium 'Smarlab BioMol-2301083' 16S ribosomal RNA gene, partial sequence.	Paenibacillus sanguinis Bacteria; Firmicutes; Bacillales; Paenibacillaceae; Paenibacillus	Undescribed bacterial pathogens isolated from human tissues, with low-score genetic identification
AF502234 Uncultured bacterium clone HP1B89 16S ribosomal RNA gene, partial sequence.	Bacteria; environmental samples.	Polyphosphate Kinase from Activated Sludge Performing Enhanced Biological Phosphorus Removal JOURNAL Appl. Environ. Microbiol. 68 (10), 4971-4978 (2002)
AF548381 Bacterium SG-3 16S ribosomal RNA gene, complete sequence.	bacterium SG-3 Bacteria; Proteobacteria; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae.	An Aquatic Bacterium That Lyses Cyanobacteria Associated with off-Flavor of Channel Catfish (*Ictalurus punctatus*)
AB161360 Lysobacter brunescens gene for 16S rRNA, partial sequence.	Lysobacter brunescens Bacteria; Proteobacteria; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae; Lysobacter.	Lysobacter concretionis sp. nov., isolated from anaerobic granules in an upflow anaerobic sludge blanket reactor JOURNAL Int. J. Syst. Evol. Microbiol. 55 (PT 3), 1155-1161 (2005) PUBMED 15879248
AY328770 Uncultured bacterium DSSD72 16S ribosomal RNA gene, partial sequence.	uncultured bacterium Bacteria; environmental samples.	Phylogenetic diversity of drinking water bacteria in a distribution system simulator JOURNAL J. Appl. Microbiol. 96 (5), 954-964 (2004) PUBMED 15078511
VCC2 Clone 6		
AJ536806 Uncultured bacterium partial 16S	uncultured bacterium Bacteria; environmental samples.	Molecular analysis of the microbial community in drainage water from a

rRNA gene, isolate cMM319-78.		magnesite mine, in the Graz Area, Austria
AJ536805 Uncultured bacterium partial 16S rRNA gene, isolate cMM319-77.	Bacteria; environmental samples.	Molecular analysis of the microbial community in drainage water from a magnesite mine, in the Graz Area, Austria
AJ536804 Uncultured bacterium partial 16S rRNA gene, isolate cMM319-75.	Bacteria; environmental samples	Molecular analysis of the microbial community in drainage water from a magnesite mine, in the Graz Area, Austria
AJ536803 Uncultured bacterium partial 16S rRNA gene, isolate cMM319-74.	Bacteria; environmental samples	Molecular analysis of the microbial community in drainage water from a magnesite mine, in the Graz Area, Austria
AJ536802 Uncultured bacterium partial 16S rRNA gene, isolate cMM319-73.	Bacteria; environmental samples	Molecular analysis of the microbial community in drainage water from a magnesite mine, in the Graz Area, Austria

VCC2 Clone 3

The closest relationship to this clone on the phylogenetic tree (Figure 4.43) was bacteria from the Betaproteobacteria including *Burkholderia* and *Ralstonia* species. *Ralstonia sp*. has been isolated previously from water, soil and activated sludge environments.

VCC2 Clone 4

The closest relations to this clone from the phylogenetic tree (Figure 4.43) were bacteria from Class Bacteriodetes, including Uncultured Bacteroides, previously isolated from filamentous microbial mats from aphotic (cave) sulfidic springs dominated by chemolithoautotrophic 'Epsilonproteobacteria'. Also the uncultured bacteria responsible for enhanced biological phosphorus removal in activated sludge and the Acetate- or Methanol-Utilizing Bacteria isolated under denitrifying conditions.

VCC2 Clone 5

The closest relations to this clone from the phylogenetic tree (Figure 4.43) were bacteria from the Gammaproteobacteria, including bacteria isolated from activated sludge performing enhanced biological phosphorus removal and *Lysobacter concretionis sp*. nov., isolated from anaerobic granules in an upflow anaerobic sludge blanket reactor. VCC2 Clone 5 is also closely related to VCC4 Clone 2 and VCC6 Clone 3.

VCC2 Clone 6

VCC2 number 6 is closely matched with uncultured bacteria isolated in a molecular analysis study of the microbial community in drainage water from a magnesite mine, in the Graz area in Austria.

VCC Sample 3 (Chicken Litter 230-300 mm)

VCC3 Clone 1

VCC3 Clone 1 was related to an uncultured bacterium isolated from the community analysis of a full-scale anaerobic bioreactor treating paper mill wastewater. VCC3 Clone 1 was also very closely related to VCC3 Clone 2.

VCC3 Clone 2

VCC3 Clone 2 was related to an uncultured bacterium isolated from the community analysis of a full-scale anaerobic bioreactor treating paper mill wastewater. VCC3 Clone 2 was also very closely related to VCC3 Clone 1.

VCC3 Clone 5

VCC3 Clone 5 was closely matched with a previously uncultured soil bacterium isolated during micro-colony cultivation on a soil substrate membrane system.

Table 4.4: Table detailing the VCC3 clones found in the chicken litter layer 230-300 mm from the surface of the reactor.

VCC3 Clone 1	Taxonomy	Origin of Blast Strain
AY426438 Uncultured bacterium clone B13	Bacteria; environmental samples.	Community analysis of a full-scale anaerobic bioreactor treating paper mill wastewater JOURNAL Syst. Appl. Microbiol. 28 (2), 175-185 (2005) PUBMED 15830810
AF050572 Uncultured eubacterium WCHB1-81	Bacteria; Actinobacteria; environmental samples.	Microbial diversity in a hydrocarbon- and chlorinated-solvent-contaminated aquifer undergoing intrinsic bioremediation JOURNAL Appl. Environ. Microbiol. 64 (10), 3869-3877 (1998) PUBMED 9758812
AF351232 Uncultured actinobacterium clone 4-42	Bacteria; Actinobacteria; environmental samples.	Diversity of 16S rDNA and naphthalene dioxygenase genes from coal-tar-waste-contaminated aquifer waters JOURNAL Microb. Ecol. 44 (2), 95-106 (2002) PUBMED 12087425
AY053493 Uncultured bacterium At425_EubF1	Bacteria; environmental samples.	Bacteria and Archaea physically associated with Gulf of Mexico gas hydrates JOURNAL Appl. Environ. Microbiol. 67 (11), 5143-5153 (2001) PUBMED 11679338
AY093472 Uncultured bacterium clone MB-B2-107	Bacteria; environmental samples	Microbial Communities from Methane Hydrate-Bearing Deep Marine Sediments in a Forearc Basin JOURNAL Appl. Environ. Microbiol. 68 (8), 3759-3770 (2002) PUBMED 12147470
VCC3 Clone 2		
AB177077 Uncultured bacterium gene	Bacteria; environmental samples	Phylogenetic diversity of subseafloor prokaryotic community associated with methane hydrate
AJ853628 Uncultured bacterium partial	Bacteria; environmental samples.	Molecular phylogenetic diversity of bacteria associated with the leachate of a closed municipal solid waste landfill JOURNAL FEMS Microbiol. Lett. 242 (2), 297-303 (2005) PUBMED 15621451
VCC3 Clone 5		
AY230768 Bacterium	Paenibacillus sanguinis	Undescribed bacterial pathogens isolated

'Smarlab BioMol-2301083' 16S ribosomal RNA gene, partial sequence.	Bacteria; Firmicutes; Bacillales; Paenibacillaceae; Paenibacillus	from human tissues, with low-score genetic identification
AF502234 Uncultured bacterium clone HP1B89 16S ribosomal RNA gene, partial sequence.	Bacteria; environmental samples.	Polyphosphate Kinase from Activated Sludge Performing Enhanced Biological Phosphorus Removal JOURNAL Appl. Environ. Microbiol. 68 (10), 4971-4978 (2002)
AF548381 Bacterium SG-3 16S ribosomal RNA gene, complete sequence.	bacterium SG-3 Bacteria; Proteobacteria; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae.	An Aquatic Bacterium That Lyses Cyanobacteria Associated with Off-Flavor of Channel Catfish (*Ictalurus punctatus*)
AB161360 *Lysobacter brunescens* gene for 16S rRNA, partial sequence.	Lysobacter brunescens Bacteria; Proteobacteria; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae; Lysobacter.	*Lysobacter concretionis sp.* nov., isolated from anaerobic granules in an upflow anaerobic sludge blanket reactor JOURNAL Int. J. Syst. Evol. Microbiol. 55 (PT 3), 1155-1161 (2005) PUBMED 15879248
AY328770 Uncultured bacterium DSSD72 16S ribosomal RNA gene, partial sequence.	uncultured bacterium Bacteria; environmental samples.	Phylogenetic diversity of drinking water bacteria in a distribution system simulator JOURNAL J. Appl. Microbiol. 96 (5), 954-964 (2004) PUBMED 15078511

VCC Sample 4 (Sawdust Layer 300cm)

Table 4.5: Table detailing the VCC4 clones found in the sawdust layer 300 mm from the surface of the reactor

VCC4 Clone 2	Taxonomy	Origin of Blast Strain
AY707568 Uncultured organism clone C4	unclassified sequences; environmental samples.	Shifts in rhizoplane communities of aquatic plants after cadmium exposure JOURNAL Appl. Environ. Microbiol. 71 (5), 2484-2492 (2005) PUBMED 15870338
AY230768 Bacterium 'Smarlab BioMol-2301083' 16S ribosomal RNA gene, partial sequence.	Paenibacillus sanguinis Bacteria; Firmicutes; Bacillales; Paenibacillaceae; Paenibacillus	Undescribed bacterial pathogens isolated from human tissues, with low-score genetic identification. Previously also identified by EBRU in borehole samples.
AF502234 Uncultured bacterium clone HP1B89 16S ribosomal RNA gene, partial sequence.	Bacteria; environmental samples.	Polyphosphate Kinase from Activated Sludge Performing Enhanced Biological Phosphorus Removal JOURNAL Appl. Environ. Microbiol. 68 (10), 4971-4978 (2002)
AF548381 Bacterium SG-3 16S ribosomal RNA gene, complete sequence.	bacterium SG-3 Bacteria; Proteobacteria; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae.	An Aquatic Bacterium That Lyses Cyanobacteria Associated with Off-Flavor of Channel Catfish *(Ictalurus punctatus*)
AB161360 Lysobacter brunescens gene for 16S rRNA, partial sequence.	Lysobacter brunescens Bacteria; Proteobacteria; Gammaproteobacteria;	Lysobacter concretionis sp. nov., isolated from anaerobic granules in an upflow anaerobic sludge blanket reactor

	Xanthomonadales; Xanthomonadaceae; Lysobacter.	JOURNAL Int. J. Syst. Evol. Microbiol. 55 (PT 3), 1155-1161 (2005) PUBMED 15879248
AY328770 Uncultured bacterium DSSD72 16S ribosomal RNA gene, partial sequence.	uncultured bacterium Bacteria; environmental samples.	Phylogenetic diversity of drinking water bacteria in a distribution system simulator JOURNAL J. Appl. Microbiol. 96 (5), 954-964 (2004) PUBMED 15078511
VCC4 Clone 3		
Y18214 *Desulfonosporus thiosulfogenes*	Desulfonispora thiosulfatigenes Bacteria; Firmicutes; Clostridia; Clostridiales; Peptococcaceae; Desulfonispora.	*Desulfonispora thiosulfatigenes* gen. nov., sp. nov., a taurine-fermenting, thiosulfate-producing anaerobic bacterium JOURNAL Int. J. Syst. Bacteriol. 49 Pt 4, 1599-1603 (1999) PUBMED 10555341
AJ852336 Uncultured bacterium partial 16S rRNA gene, clone MKEW-5.	Bacteria; environmental samples	Structure and Topology of Microbial Communities in the Major Gut Compartments of *Melolontha melolontha* Larvae (Coleoptera: Scarabaeidae) JOURNAL Appl. Environ. Microbiol. 71 (8), 4556-4566 (2005) PUBMED 16085849
AF159120 *Desulfosporosinus sp*. 5apy	*Desulfosporosinus sp*. 5apy Bacteria; Firmicutes; Clostridia; Clostridiales; Peptococcaceae; Desulfosporosinus.	Sulfate reducing bacteria in Berlin raw and drinking water
AY684082 Uncultured *Clostridium sp*. clone MRE50b26	uncultured *Clostridium sp.* Bacteria; Firmicutes; Clostridia; Clostridiales; Clostridiaceae; Clostridium; environmental samples.	Retrieval of first genome data for rice cluster I methanogens by a combination of cultivation and molecular techniques JOURNAL FEMS Microbiol. Ecol. 53 (2), 187-204 (2005)
U85418 Unidentified Clostridiaceae pDH-C	unidentified Clostridiaceae pDH-C Bacteria; Firmicutes; Clostridia; Clostridiales; environmental samples.	Direct Submission

VCC4 Clone 2

The closest relations to this clone from the phylogenetic tree (Figure 4.43) were bacteria from the Gammaproteobacteria, including bacteria isolated from activated sludge performing enhanced biological phosphorus removal and *Lysobacter concretionis sp*. nov., isolated from anaerobic granules in an upflow anaerobic sludge blanket reactor. VCC4 Clone 2 is also closely related to VCC2 Clone 5 and VCC6 Clone 3, and may be the same organism.

VCC4 Clone 3

VCC4 clone 3 was closely matched to *Desulfosporosinus sp*., a sulfate reducing bacterium found in Berlin raw and drinking water.

VCC Sample 5 (Woodchip Layer 300-830 mm)

VCC5 Clone 1

VCC5 clone 1 was found to be closely matched to bacteria isolated from methanogenic communities in a northern raised bog complex and to an uncultured Acidobacterium isolated in microbial mats in a chemoautotrophic cave.

Table 4.6: Table detailing the VCC5 clones found in the Woodchip layer 300-830 mm from the surface of the reactor

VCC5 Clone 1	Taxonomy	Origin of Blast Strain
AF523981Uncultured bacterium clone FW34	uncultured bacterium Bacteria; environmental samples	Recovery of novel bacterial diversity from a forested wetland impacted by reject coal JOURNAL Environ. Microbiol. 4 (11), 764-769 (2002) PUBMED 12460285
AM180890Uncultured Acidobacteria bacterium partial 16S rRNA gene, clone SS_LKC22_UB76.	uncultured Acidobacteria bacterium Bacteria; Acidobacteria; environmental samples.	In situ detection of novel Acidobacteria in microbial mats in a chemoautotrophic cave (Lower Kane Cave, WY, USA) by means of an extensive 16S rRNA and 23S rRNA multiple probe concept
AB113590 Uncultured Clostridiales bacterium gene for 16S rRNA, partial sequence, clone: HAuD-UB36.	uncultured Clostridiales bacterium Bacteria; Firmicutes; Clostridia; Clostridiales; environmental samples.	Changes in microbial community and biogeochemical process along a subsurface geothermal water stream in a Japanese gold mine
AB113614Uncultured Clostridiales bacterium gene for 16S rRNA, partial sequence, clone: HAuD-LB9.	uncultured Clostridiales bacterium Bacteria; Firmicutes; Clostridia; Clostridiales; environmental samples.	Changes in microbial community and biogeochemical process along a subsurface geothermal water stream in a Japanese gold mine
AJ853816Uncultured bacterium partial 16S rRNA gene, clone MES-16.	Bacteria; environmental samples.	Methanogen communities and Bacteria along an ecohydrological gradient in a northern raised bog complex

VCC Sample 6 (Hay/Chicken Layer 830-890 mm).

Table 4.7: Table detailing the VCC6 clones found in the Hay / Chicken Litter layer 830-890 mm from the surface of the reactor

VCC6 Clone 1	Taxonomy	Origin of Blast Strain
DQ060410Uncultured bacterium clone F21 16S ribosomal RNA gene, partial sequence.	Bacteria; environmental samples.	RFLP analysis of AOB-specific 16S rDNA library from red soil enrichment culture
AY566234Uncultured bacterium clone PCF1 16S ribosomal RNA gene, partial sequence.	Bacteria; environmental samples.	Microcolony Cultivation on a Soil Substrate Membrane System Selects for Previously Uncultured Soil Bacteria JOURNAL Appl. Environ. Microbiol. 71 (12), 8714-8720 (2005)

AB234250Uncultured bacterium gene for 16S rRNA, partial sequence, clone:LS4-178.	Bacteria; environmental samples.	Biotransformation of Polychlorinated Dioxins and Microbial Community Dynamics in Sediment Microcosms at Different Contamination Levels JOURNAL Microb. Environ. 20, 227-242 (2006)
AB127667 Uncultured bacterium gene for 16S rRNA, partial sequence, clone:KTS60.	uncultured bacterium Bacteria; environmental samples.	Comparative analysis of bacterial diversity in freshwater sediment of a shallow eutrophic lake by molecular and improved cultivation-based techniques JOURNAL Appl. Environ. Microbiol. 71 (4), 2162-2169 (2005)
AY486133 *Methylovorus glucosetrophus* strain 6B1 16S ribosomal RNA gene,	*Methylovorus glucosetrophus* Bacteria; Proteobacteria; Betaproteobacteria; Methylophilales; Methylophilaceae; Methylovorus.	Phylogenetic position and emended description of the genus Methylovorus JOURNAL Int. J. Syst. Bacteriol. 55 (PT 2), 903-906 (2005) PUBMED 15774683
AF351574 Uncultured beta proteobacterium clone NJ76 16S ribosomal RNA gene, partial sequence.	uncultured beta proteobacterium Bacteria; Proteobacteria; Betaproteobacteria; environmental samples.	Genetic Diversity of the Beta-Subclass Ammonia-Oxidizing Bacteria in the Lake Sediment as Determined by the Sequence Analysis of 16S rRNA and amoA Genes
VCC6 Clone 3		
AY230768 Bacterium 'Smarlab BioMol-2301083' 16S ribosomal RNA gene, partial sequence.	Paenibacillus sanguinis Bacteria; Firmicutes; Bacillales; Paenibacillaceae; Paenibacillus	Undescribed bacterial pathogens isolated from human tissues, with low-score genetic identification
AF502234 Uncultured bacterium clone HP1B89 16S ribosomal RNA gene, partial sequence.	Bacteria; environmental samples.	Polyphosphate Kinase from Activated Sludge Performing Enhanced Biological Phosphorus Removal JOURNAL Appl. Environ. Microbiol. 68 (10), 4971-4978 (2002)
AF548381 Bacterium SG-3 16S ribosomal RNA gene, complete sequence.	bacterium SG-3 Bacteria; Proteobacteria; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae.	An Aquatic Bacterium That Lyses Cyanobacteria Associated with Off-Flavor of Channel Catfish (*Ictalurus punctatus*)
AB161360 *Lysobacter brunescens* gene for 16S rRNA, partial sequence.	*Lysobacter brunescens* Bacteria; Proteobacteria; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae; Lysobacter.	*Lysobacter concretionis sp.* nov., isolated from anaerobic granules in an upflow anaerobic sludge blanket reactor JOURNAL Int. J. Syst. Evol. Microbiol. 55 (PT 3), 1155-1161 (2005) PUBMED 15879248
AY328770 Uncultured bacterium DSSD72 16S ribosomal RNA gene, partial sequence.	uncultured bacterium Bacteria; environmental samples.	Phylogenetic diversity of drinking water bacteria in a distribution system simulator JOURNAL J. Appl. Microbiol. 96 (5), 954-964 (2004)

VCC6 Clone 1

VCC6 clone 1 was found to be closely matched to an uncultured bacterium previously isolated from a red soil enrichment culture. The clone was also closely related to bacteria from the Betaproteobacteria group and ammonia-oxidizing bacteria isolated from lake sediment.

VCC6 Clone 3

VCC6 clone 3 was quite similar to an uncultured bacterium isolated from activated sludge performing enhanced biological phosphorus removal. Other relations to this clone as from the phylogenetic tree (Figure 4.43) were bacteria from the Gammaproteobacteria, including *Lysobacter concretionis sp*. nov., isolated from anaerobic granules in an upflow anaerobic sludge blanket reactor. VCC6 Clone 3 is also closely related to VCC2 Clone 5 and VCC4 Clone 2.

VCC Sample 7 (Woodchips/Manure Layer 890-1130 mm)

Table 4.8: Table detailing the VCC7 clones found in the Woodchips / Manure layer 890-1130 mm from the surface of the reactor

VCC7 Clone 5	Taxonomy	Origin of Blast Strain
X93027 *Mycobacterium sp.* 16S ribosomal RNA (strain MCRO 16).	*Mycobacterium sp.* Bacteria; Actinobacteria; Actinobacteridae; Actinomycetales; Corynebacterineae; Mycobacteriaceae; Mycobacterium.	Two-laboratory collaborative study on identification of mycobacteria: molecular versus phenotypic methods JOURNAL J. Clin. Microbiol. 34 (2), 296-303 (1996) PUBMED 8789004
X93031 *Mycobacterium sp.* 16S ribosomal RNA (strain MCRO 24).	*Mycobacterium sp.* Bacteria; Actinobacteria; Actinobacteridae; Actinomycetales; Corynebacterineae; Mycobacteriaceae; Mycobacterium.	Two-laboratory collaborative study on identification of mycobacteria: molecular versus phenotypic methods JOURNAL J. Clin. Microbiol. 34 (2), 296-303 (1996) PUBMED 8789004
DQ157760 *Mycobacterium arupense* strain AR30097	*Mycobacterium arupense* Bacteria; Actinobacteria; Actinobacteridae; Actinomycetales; Corynebacterineae; Mycobacteriaceae; Mycobacterium.	*Mycobacterium arupense* sp. nov., a novel moderately growing, nonchromogenic bacterium related to M. nonchromogenicum and isolated from clinical specimens
AY360325 *Mycobacterium sp*. NIPHL130302	*Mycobacterium sp.* NIPHL130302 Bacteria; Actinobacteria; Actinobacteridae; Actinomycetales; Corynebacterineae; Mycobacteriaceae; Mycobacterium.	Xu,J., Stanley,T., Millar,B.C. and Moore,J.E. TITLE Mycobacterium sp. partial 16S rRNA gene sequence
DQ058406 *Mycobacterium nonchromogenicum* strain ATCC 19530	*Mycobacterium nonchromogenicum* Bacteria; Actinobacteria; Actinobacteridae; Actinomycetales; Corynebacterineae; Mycobacteriaceae; Mycobacterium.	Cloud,J.L., Meyer,J., Carroll,K.C., Pounder,J.I. and Woods,G.L. TITLE Characterization of a clinical isolate related to the Mycobacterium terrae complex

VCC7 Clone 6		
AB032351 *Acetobacter lovaniensis*	*Acetobacter lovaniensis* Bacteria; Proteobacteria; Alphaproteobacteria; Rhodospirillales; Acetobacteraceae; Acetobacter	Taxonomic study of the genus Acetobacter with descriptions of *Acetobacter indonesiensis sp*. nov., Acetobacter *tropicalis sp*. nov., *Acetobacter estunensis* (ex Carr 1958) nom. rev., *Aetobacer lovaniensis* (ex Frateur 1950) nom. rev., *Acetobacter orleanensis* (ex Henneberg 1906) nom rev., and *Acetobacter peroxydans* (ex Visser't Hooft 1925) nom. rev
AJ419837 *Acetobacter lovaniensis* 16S rRNA gene, strain LMG 1617.	*Acetobacter lovaniensis* Bacteria; Proteobacteria; Alphaproteobacteria; Rhodospirillales; Acetobacteraceae; Acetobacter.	Re-examination of the genus Acetobacter, with descriptions of *Acetobacter cerevisiae* sp. nov. and *Acetobacter malorum* sp. nov JOURNAL Int. J. Syst. Evol. Microbiol. 52 (Pt 5), 1551-1558 (2002) PUBMED 12361257
AB180930 *Acetobacter orientalis* gene for 16S ribosomal RNA, partial sequence.	*Acetobacter orientalis* Bacteria; Proteobacteria; Alphaproteobacteria; Rhodospirillales; Acetobacteraceae; Acetobacter.	Identification and characterization of lactococcal and Acetobacter strains isolated from traditional Caucasusian fermented milk JOURNAL J. Nutr. Sci. Vitaminol. 51, 187-193 (2005)
AB219054 *Acetobacter sp*. A01 gene for 16S ribosomal RNA, partial sequence.	*Acetobacter sp*. A01 Bacteria; Proteobacteria; Alphaproteobacteria; Rhodospirillales; Acetobacteraceae; Acetobacter.	Succession of bacterial and fungal communities during a traditional pot fermentation of rice vinegar (TSUBOZU) assessed by PCR-mediated denaturing gradient gel electrophoresis
AB052709 *Acetobacter orientalis* gene for 16S rRNA, strain:1B-M18A.	*Acetobacter orientalis* Bacteria; Proteobacteria; Alphaproteobacteria; Rhodospirillales; Acetobacteraceae; Acetobacter.	Identification of Acetobacter strains from Indonesian sources, and proposal of *Acetobacter roseisyzygii* sp. nov., *Acetobacter cibinongensis* sp. nov., and *Acetobacter orientalis* sp. nov

VCC7 clone 5

VCC7 clone 5 is closely related to Mycobacterium sp., a member of the Actinobacteria, it a novel, moderately growing, non-chromogenic bacterium isolated from clinical specimens.

VCC7 clone 6

VCC7 clone 6 is matched with *Acetobacter lovaniensis* and *Acetobacter orientalis.* These bacteria are members of the Alphaproteobacteria and have been isolated from the fermentation of milk and rice vinegar.

VCC Sample 11 (Hay Layer 1200-1420 mm)

Table 4.9: Table detailing the VCC11 clones found in the Hay layer 1200-1420 mm from the surface of the reactor.

VCC11 Clone 1	Taxonomy	Origin of Blast Strain
AY426472Uncultured bacterium clone E8	Bacteria; environmental samples.	Community analysis of a full-scale anaerobic bioreactor treating paper mill wastewater

		JOURNAL Syst. Appl. Microbiol. 28 (2), 175-185 (2005) PUBMED 15830810
AF282178Uncultured bacterium Btol	uncultured bacterium Btol Bacteria; Proteobacteria; Deltaproteobacteria; environmental samples.	*Desulfomonile limimaris* sp. nov., an anaerobic dehalogenating bacterium from marine sediments JOURNAL Int. J. Syst. Evol. Microbiol. 51 (Pt 2), 365-371 (2001) PUBMED 11321081
AB244318Uncultured bacterium gene for 16S ribosomal RNA, partial sequence, clone:LCFA-B11.	Bacteria; environmental samples.	Microbial diversity of a mesophilic long-chain fatty acids-degrading methanogenic consortium in chemostat cultivation
AY167450Uncultured *Desulfomonile sp*. clone SbIDsmon4	uncultured *Desulfomonile sp.* Bacteria; Proteobacteria; Deltaproteobacteria; Syntrophobacterales; Syntrophaceae; Desulfomonile; environmental samples.	Microarray and functional gene analyses of sulfate-reducing prokaryotes in low-sulfate, acidic fens reveal cooccurrence of recognized genera and novel lineages JOURNAL Appl. Environ. Microbiol. 70 (12), 6998-7009 (2004) PUBMED 15574893
AM086646 *Desulfomonile tiedjei* partial 16S rRNA gene, strain DSM 6799.	*Desulfomonile tiedjei* Bacteria; Proteobacteria; Deltaproteobacteria; Syntrophobacterales; Syntrophaceae; Desulfomonile.	Vertical distribution and activity of sulfate reducing bacteria belonging to the genus Desulfomonile in the anoxic layers of the meromictic Lake Cadagno

VCC11 clone 1

VCC11 clone 1 was found to be closely related to an uncultured bacterium from a full-scale anaerobic bioreactor treating paper mill wastewater.

4.7 CONCLUSIONS

The SRU sampling operation was executed successfully and, with the exception of the sawdust layers, it proved possible to identify all the other packing layers in the core sample from the as-built plans. Both water samples and reactor core samples were recovered, and based on analysis of these, it is possible to draw provisional conclusions relating to the function and possibly also the failure of the conventional SRU packed bed reactor. These observations are considerably strengthened when compared to the studies of lignocellulose packed-bed columns undertaken previously as controlled laboratory studies (Clarke, 2006).

While it was anticipated that a substantial portion of the lignocellulose complex of the woodchip fraction would remain present in the system, even after a number of years of reactor operation, it was surprising to note that even the soluble wood extractives fraction still remained as a substantial component of the woodchip sample. Together with the high residual levels of pectin observed, this indicates extremely poor utilization of the substrate. These observations are largely supported by the electron microscopy study. It should also be noted that wood particle size plays a role, and that sawdust was more degraded than woodchips over the course of reactor operation.

The SEM results of the woodchip samples did, however, show some differentiation between the Top, Middle and Bottom layers, which was also generally supported by the water sample analyses. It was apparent that the upper layer was more degraded than the woodchip layers lower down in the reactor. Consumption of aromatic compounds, TOC and VFA was also apparently higher in the upper layer. Elevated reducing sugars in this zone also suggested enhanced breakdown of the lignocellulose complex here.

The above results accord well with the Degrading Packed Bed Reactor (DPBR) hypothesis which argues that high activity in the upper layer is responsible for sustaining the activity of the full column. However when the easily available substrate for sulfate reduction becomes exhausted in this zone, the whole process gradually unwinds. This corresponds with the picture that emerges and accounts in part for the poor performance observed.

The phylogenetic study provides an interesting observation of the presence of many of the bacterial physiological types that have been associated with the successful performance of the DPBR laboratory packed-bed reactor. These studies, undertaken under controlled laboratory conditions, have provided a structured outline of the microbial processes occurring in these systems (Clarke, 2006).

The observation of comparability of the two results provides a very useful indication that the failure of the SRU system was not due to the lack of appropriate biocatalytic genetic potential. On the contrary, most of the necessary forms were found to be present. It may thus be concluded, as a confirmatory follow-up to previous studies, that the lignocellulose packed-bed system is inherently unstable. Without some form of external supplementation to maintain sulfide production in the system, the accessibility of the lignocellulose complex, and even the pectin and more easily extractible fractions, will decline with time.

The results reported here provide an important supportive contribution to the operational hypothesis on which the DPBR reactor design concept is based.

4.8 MINERALOGICAL AND GEOCHEMICAL STUDY

XRD analysis, solid geochemistry by XRF analysis and a microscopy study were used to perform the mineralogical study.

4.8.1 Mineralogy

The mineralogical study included semi-quantitative analysis by XRD analysis and a microscopy study. The mineral compositions determined by XRD are tabulated in Table 4.10 and plotted in diagrams with trend lines added (Figure 4.44). The major mineral components, identified by XRD included:

- Pyrite (FeS_2);
- K-feldspar ($KAlSi_3O_8$).
- Illite ($K_{1.5\text{-}1.0}Al_4[Si_{6.5\text{-}7.0}Al_{1.5}\text{-}1.0O_{20}](OH)_4$ and smectite (di-octahedral: 0.5Ca, $Na)_{0.7}(Al,Mg,Fe_4)[Si,Al]_8O_{20})(OH)_4nH_2O$; Tri-octahedral: $(0.5Ca,Na)_{0.7}(Mg,Fe,Al)_6[(Si,Al)_8O_{20}(OH)_4nH_2O)$.

- Quartz (SiO_2).
- Gypsum ($CaSO_4 2H_2O$) is the major second mineral containing sulfur in its structure.
- Other minerals including Cu, Pb, Zn, Mn.

Table 4.10: Summary of mineral compositions determined by XRD.

Sample name	Abbreviation	Pyrite	K-feldspar	Quartz	Gypsum	Illite/smectite
Sewage sludge/ chicken litter mix	SCM mix	4.00	-	86.00	-	10.00
Woodchips top	WC top	6.00	-	83.00	3.00	8.00
Manure	Manure	3.00	-	87.00	4.00	6.00
Woodchips mid	WC mid	5.00	-	81.00	3.00	11.00
Woodchips bot	WC bot	6.00	8.00	76.00	3.00	7.00
Manure+hay mix	M+H mix	1.00	3.00	93.00	1.00	2.00
Hay	Hay	2.00	6.00	84.00	2.00	6.00

The results clearly indicated the following (Figure 4.44 and Table 4.10):

- Pyrite is the major sulfide mineral containing sulfur in its mineral structure. It has precipitated in the bioreactor and decreases in concentration towards the bottom (effluent). This trend is certainly related to chemical reaction rather than deposition due to gravity. Microscopy study has indicated that pyrite is enriched in all the samples and occurs as extremely fine-grained elongated, needle-like grains (Figure 4.45). Occasionally, some larger grains can be found in thin sections.
- In general, illite/smectite followed a similar trend as found for pyrite. Illite/smectite is a type of typical clay mineral that has a large capacity to adsorb trace metals onto its surface. It is unsure as to which metals can be adsorbed by illite/smectite in the bioreactor in this study. Further studies may be required if the detailed mechanisms are required.
- Quartz and K-feldspar also followed a similar trend, *i.e*. increasing deposition towards the bottom (effluent). Two types of quartz were identified including detrital (primary) quartz and chalcedony quartz (secondary). Silification (chalcedony mineralisation) increased towards the bottom of SSRU1.
- Gypsum is the major mineral present and contains sulphite in its mineral structure. Although gypsum wasn't detected through XRD in the top section (influent), the overall trend indicated a clear decrease in gypsum concentration along the depth of SSRU1. It is clear that gypsum is a secondary product of chemical or biochemical reaction rather than a primary mineral by deposition due to gravity. Gypsum ($CaSO_4 2H_2O$) has precipitated and is often seen in all the samples as a fresh secondary mineral.
- Minerals containing Pb, Cu, Zn were found and might be galena (PbS), chalcopyrite ($CuFeS_2$), sphalerite (ZnS). However, further studies are required to confirm this as these minerals were only found in trace concentrations.
- Manganese minerals (MnO) precipitated on the surface, or cells, fibres of bioreactor materials, *e.g*. wood chips (Figure 4.47).
- Trace elements including Rb, Sr, Y, Ba may replace Ca as trace solid solution to Ca in gypsum or other Ca containing minerals, *e.g.* feldspar; and
- Other trace heavy metals including Ni, V, Cr, Co might occur as trace minerals, adsorbed on the surface, cells, or fibres of bioreactor materials like wood chips (Figure 4.47).

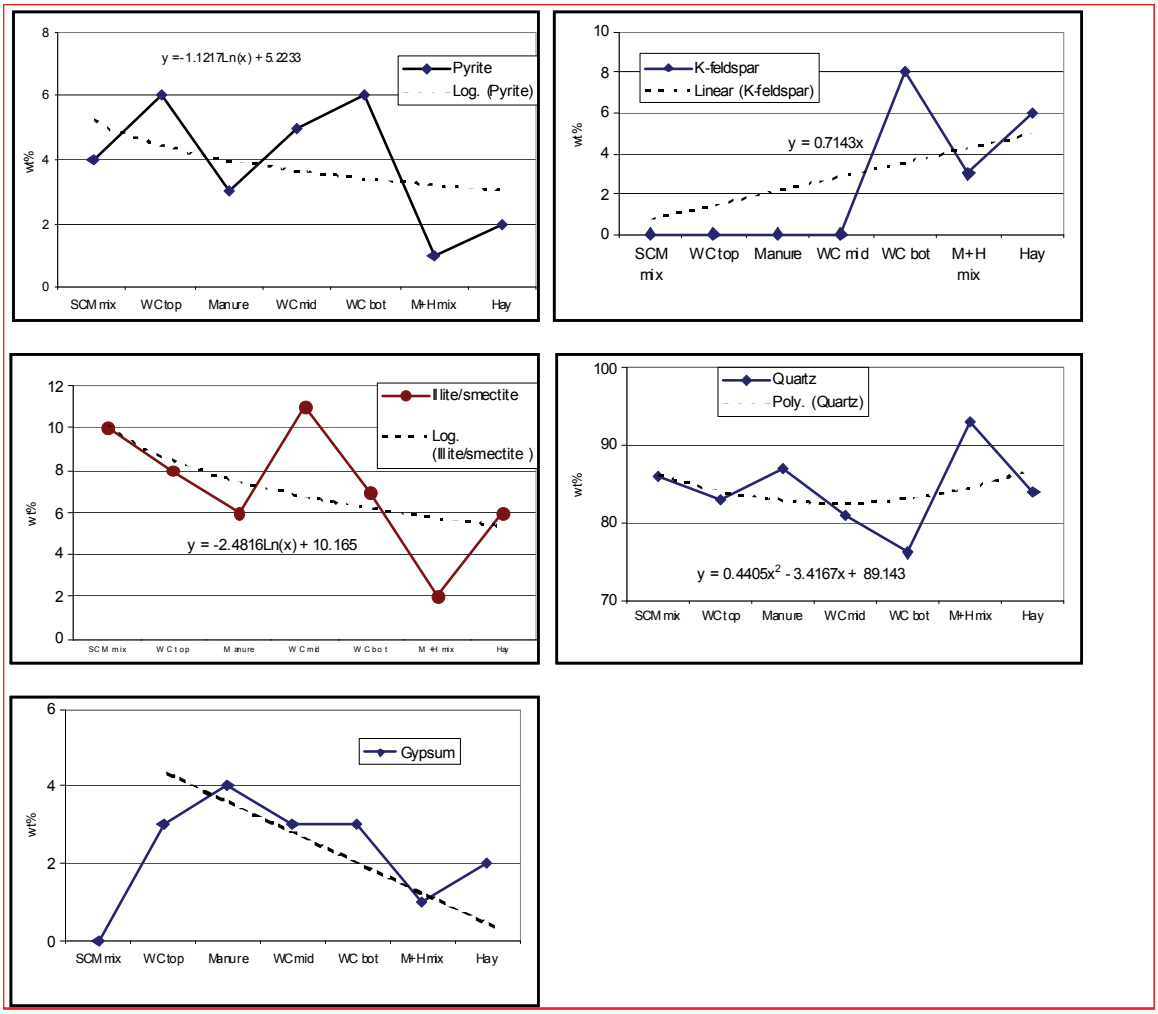


Figure 4.44: Mineral distribution against locality from top (influent) to bottom (effluent).

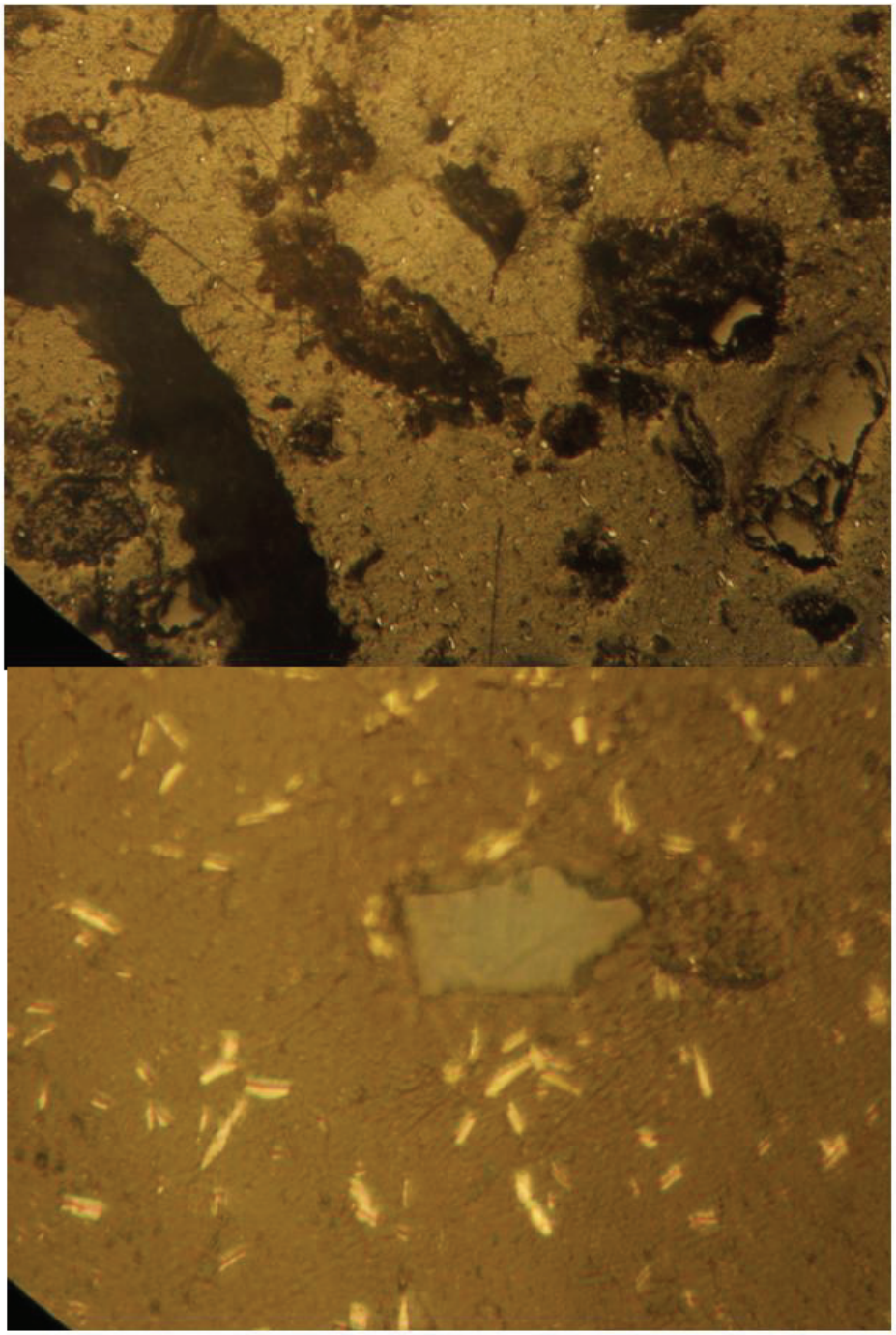


Figure 4.45: Fine-grained secondary pyrite, 40x (top), 100 x (bottom).

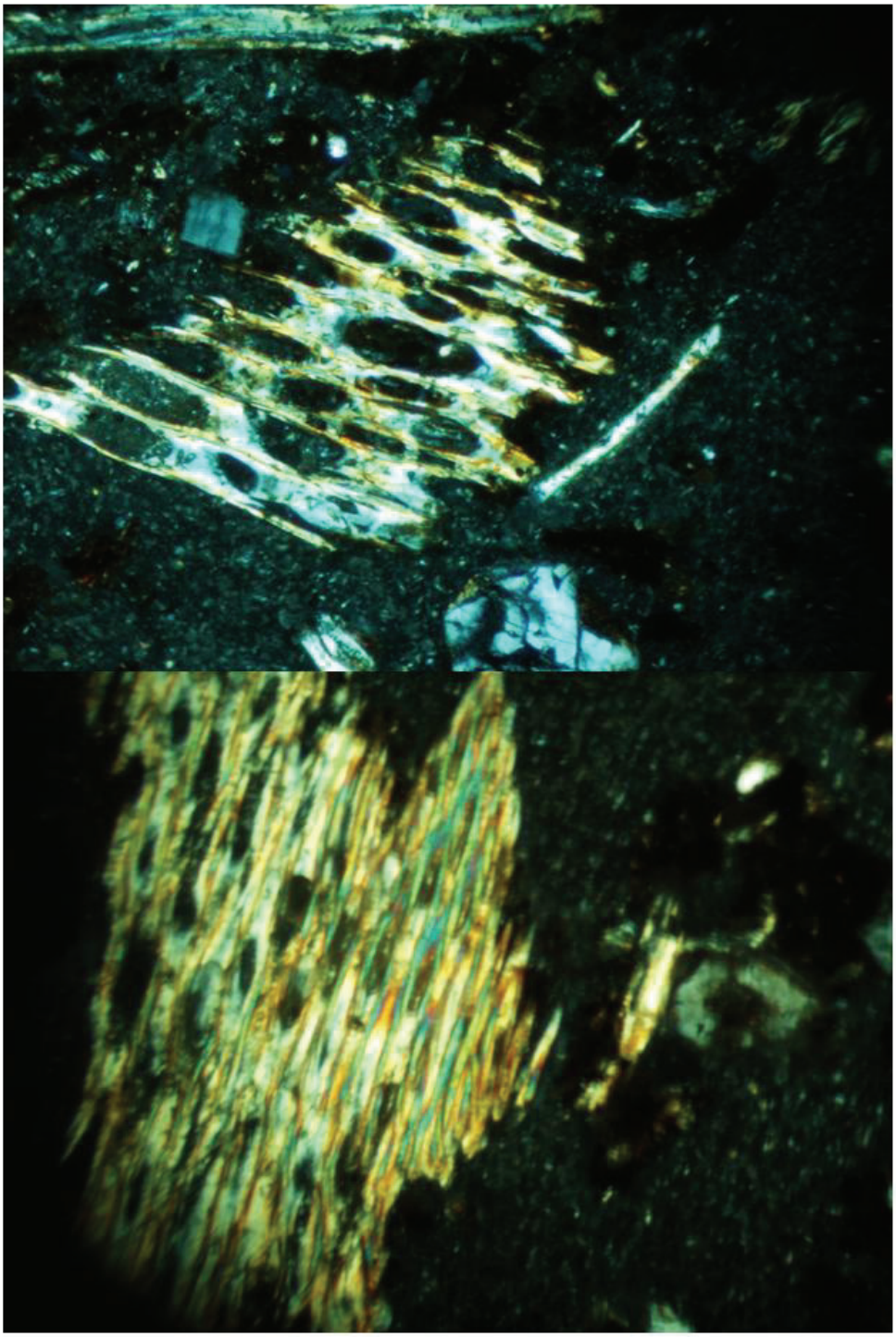


Figure 4.46: precipitates along wood cells, silicification (top), illite/smectite (bottom), 40x.

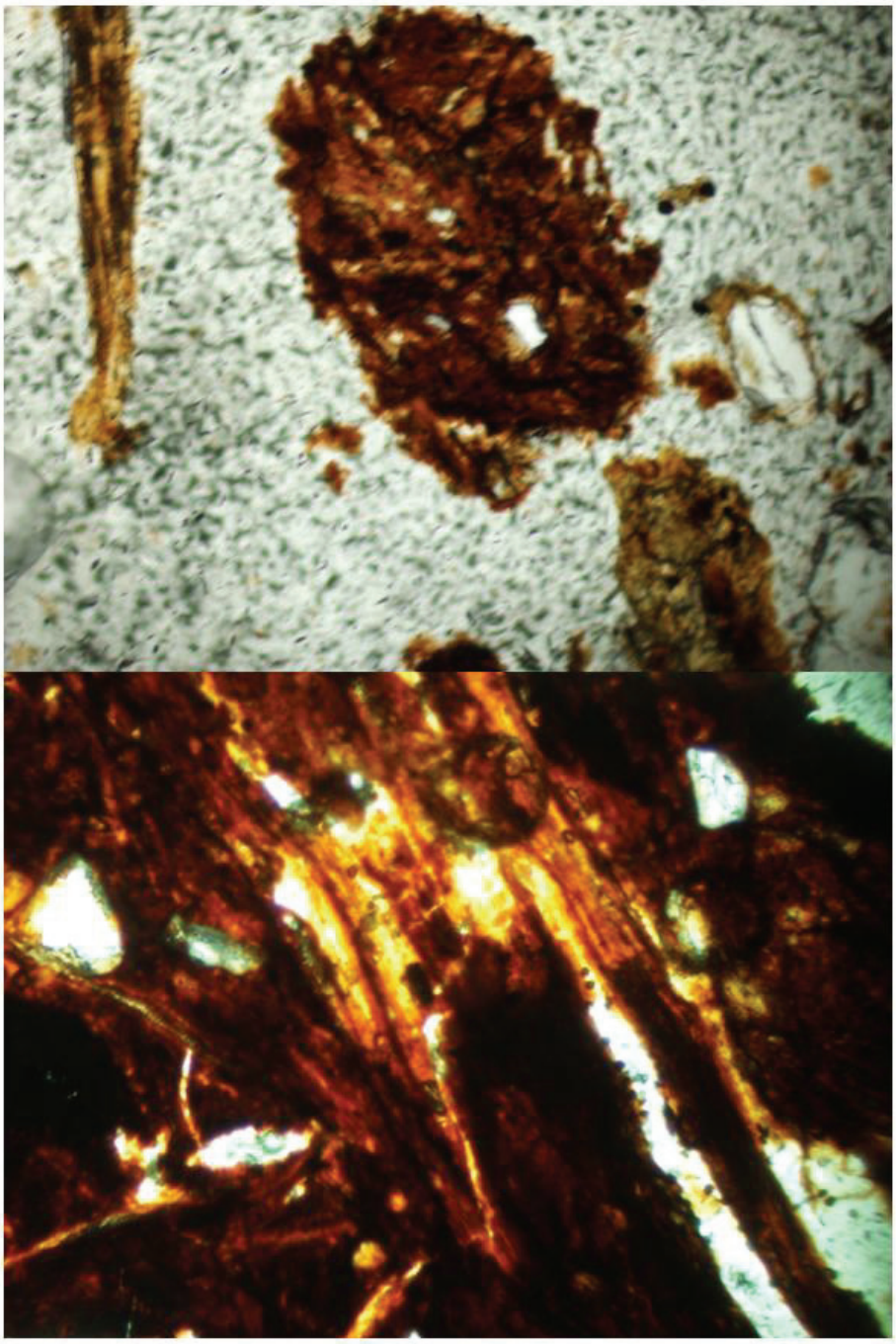


Figure 4.47: Mn adsorption (red) on wood chips and silicification (white) along wood fibres, 40x (top), 100x (bottom).

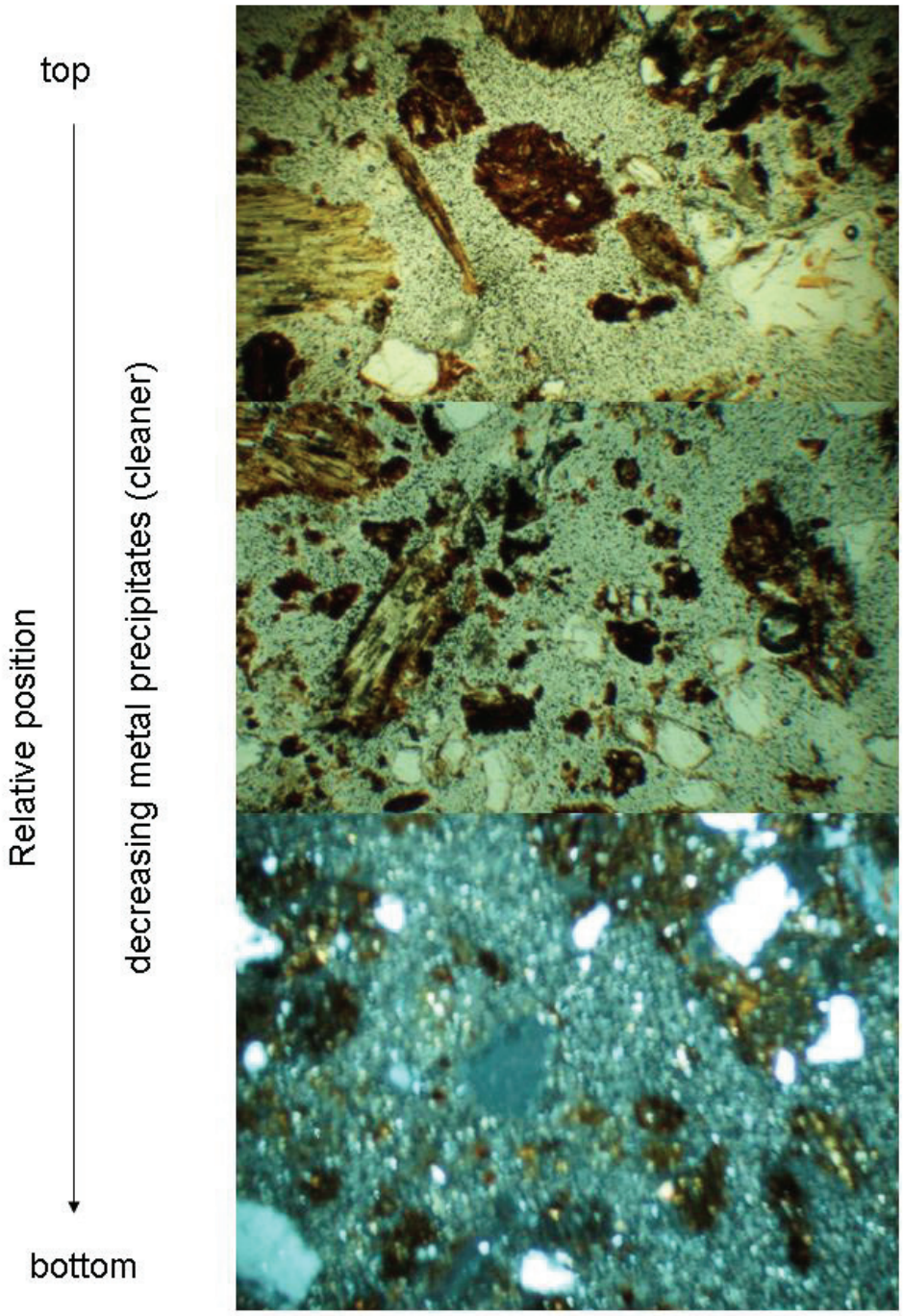


Figure 4.48: Decreasing metal precipitation and adsorption on wood chips from top (influent) to bottom (effluent), about 100x.

4.8.2 Solid geochemistry

Solid geochemistry has been determined by XRF in terms of major oxides in weight percentage (wt%) and trace metals in parts per million (ppm). The geochemical results are tabulated in Table 4.10 and also presented in Figure 4.49 showing the distribution from top (influent) to bottom (effluent). The results for major elements (oxides) can be summarised as follows:

- Fe_2O_3 and S chemical concentrations significantly decreasing from top to bottom, which is in line gypsum, specifically with pyrite;
- Al_2O_3 chemical concentration decreasing from top to bottom, which is in line with clay minerals like illite/smectite;
- Na2O chemical concentration decreasing from top to bottom, which might be related to Na_2SO_4 or halite. It might not be related with illite/smectite;
- SiO_2, and K_2O concentrations decreasing from top to bottom in general, which are in line with quartz and illite/smectite respectively.

The results for trace elements can be summarised as follows:

- Co, Ni, Cu, Zn, Pb concentrations significantly decreasing from top to bottom.
- Cr, V concentrations decreasing, but increasing towards bottom, which needs to further be investigated; and
- No clear trend for Ti and other elements (Figure 4.50).

4.8.3 Conclusions

The results clearly indicated the following:

- Pyrite is the major sulfide mineral containing S. It has precipitated in the bioreactor and decreases in concentration towards the bottom (effluent), which is in line with Fe_2O_3 and S;
- Gypsum is the major sulfate mineral containing S. It decreases in concentration from top to bottom in general, which doesn't coincide with Ca.
- Quartz deposition increased towards bottom (effluent), specifically with chalcedony, which is in line with SiO_2 chemical concentration;
- Manganese minerals (MnO) precipitate on the surface, or cells, fibres of bioreactor materials, *e.g.* wood chips;
- Al_2O_3 chemical concentration decreased from top to bottom of SSRU1, which is in line with clay minerals like illite/smectite;
- Na_2O chemical concentration decreased from top to bottom of SSRU1, which might be related to Na_2SO_4 or halite. It might not be related with illite/smectite;
- In general, SiO_2, and K_2O concentrations decreased from top to bottom which are in line with quartz and illite/smectite respectively;

Table 4.11: Solid geochemistry determined by XRF (Major elements).

	Major (wt%)												
Sample	**Abbreviation**	**SiO_2**	**TiO_2**	**Al_2O_3**	**Fe_2O_3**	**MnO**	**MgO**	**CaO**	**Na_2O**	**K_2O**	**P_2O_5**	**LOI**	**Total**	**S**
Sewage sludge/chicken litter mixture	SCM mix	35.60	0.43	7.70	4.75	0.06	1.23	0.81	0.42	0.57	0.42	44.05	96.04	5.50
Woodchips top	WC top	40.81	0.47	5.51	6.34	0.14	0.31	1.37	0.18	0.64	1.00	40.75	97.52	1.53
Manure	Manure	34.86	0.56	7.45	6.03	0.18	0.60	1.72	0.31	0.66	0.67	45.81	98.85	3.20
Woodchips middle	WC mid	4.01	0.16	2.08	2.93	0.07	0.20	0.72	0.06	0.20	0.43	77.89	97.75	0.84
Manure & hay mixture	M+H mix	51.05	0.55	4.69	3.50	0.12	0.28	0.86	0.27	0.72	0.19	37.10	99.33	2.50
Hay	Hay	44.98	0.51	4.92	4.06	0.13	0.58	1.95	0.20	0.69	0.47	38.85	97.34	2.00

Table 4.12: Solid geochemistry determined by XRF (Trace elements).

	ppm														
Sample	**Abbreviation**	**SiO2**	**Rb**	**Sr**	**Y**	**Zr**	**Nb**	**Co**	**Ni**	**Cu**	**Zn**	**Ti**	**V**	**Cr**	**Ba**	**Pb**
Sewage sludge/chicken litter mixture	SCM mix	35.60	45	117	83	201	6	214	547	206	1689	3361	67	79	439	101
Woodchips top	WC top	40.81	45	206	63	245	6	120	283	77	489	3121	73	64	322	32
Manure	Manure	34.86	44	304	62	216	6	123	255	335	1095	3842	74	6	43	13
Woodchips mid	WC mid	4.01	16	126	32	76	<3	90	153	68	284	1140	27	29	173	38
Manure & hay mixture	M+H mix	51.05	30	104	22	180	4	26	44	24	70	2281	55	44	197	10
Hay	Hay	44.98	45	170	29	252	7	41	69	39	118	3481	88	73	299	15

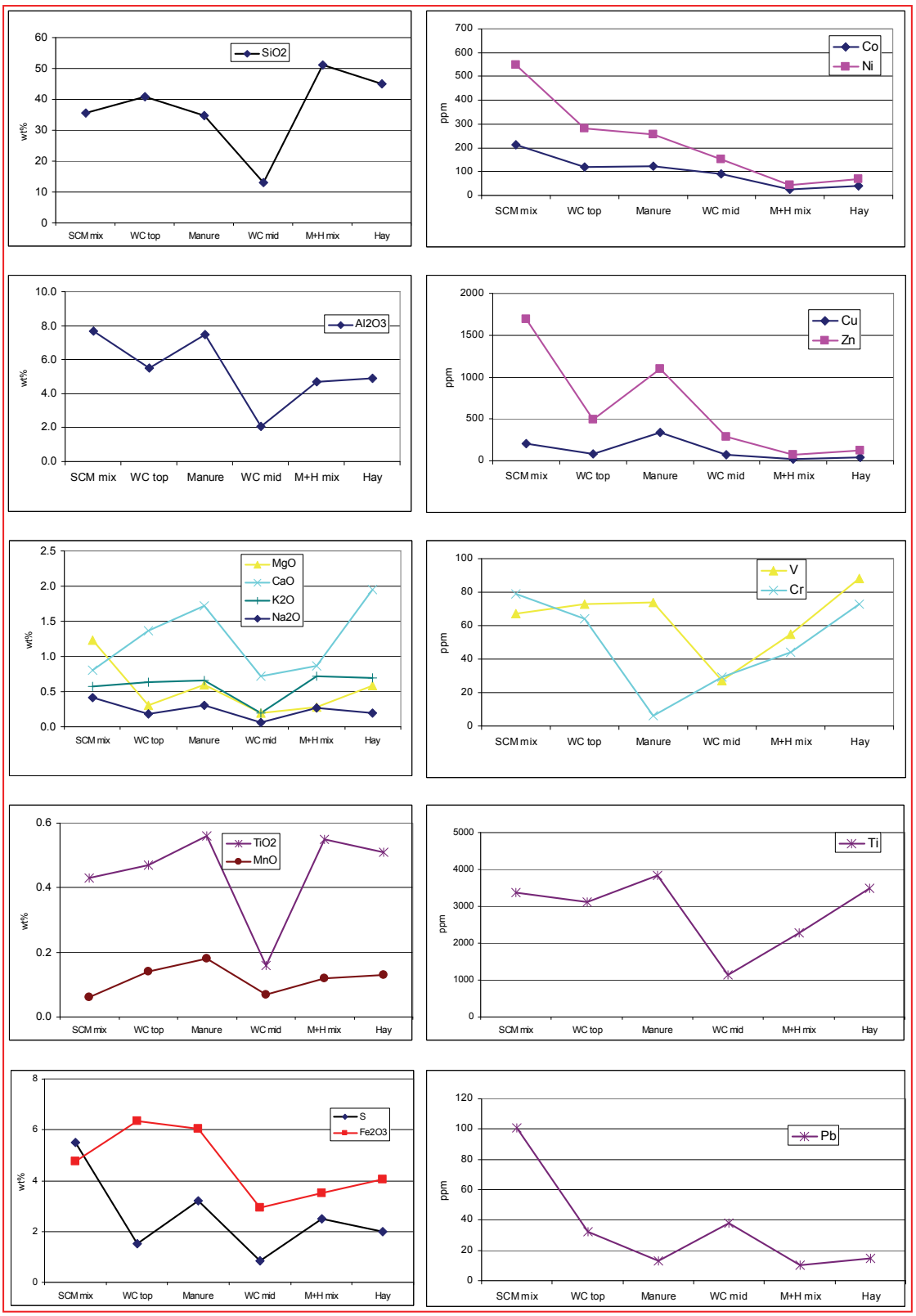


Figure 4.49: Chemical composition distribution from top (influent) to bottom (effluent).

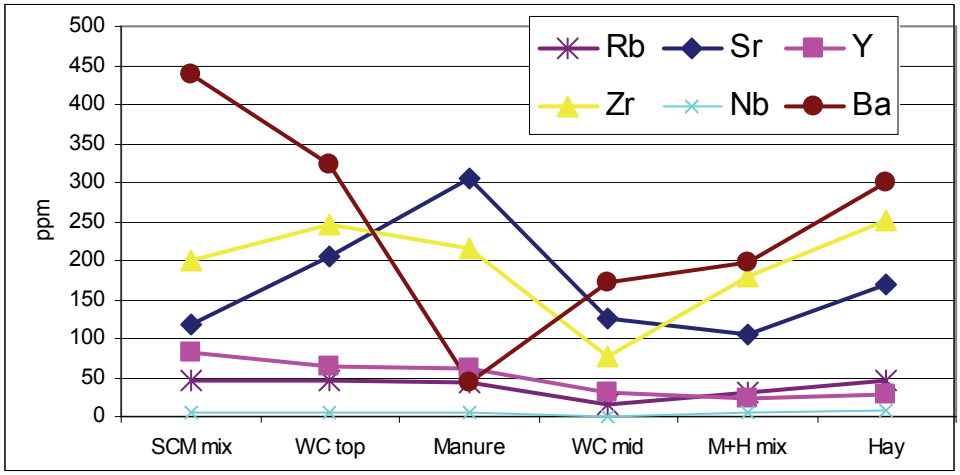


Figure 4.50: Chemical concentrations of Rb, Sr, Y, Zr, Nb, Ba.

CHAPTER 5
EVALUATION OF THE HYDRAULIC PERFORMANCE OF THE SULFATE REDUCING UNITS

5.1 INTRODUCTION

In order to understand the hydraulics of the DPBR, SSRU2 was selected as a control and sodium bromide was used as a tracer to generate tracer response curves for the units.

5.2 TRACER STUDIES

The tracer response curve is drawn from the time it takes for the sodium bromide to be detected in the effluent after injection into the feed. The time measured would be compared to the predicted response curve (based on the theoretical retention time of the units) using the "CSTRs-in-series model" (Appendix A). The predicted response curve is presented in Figure 5.1 with the sodium bromide peak recorded at 68 hours (Figure 5.1).

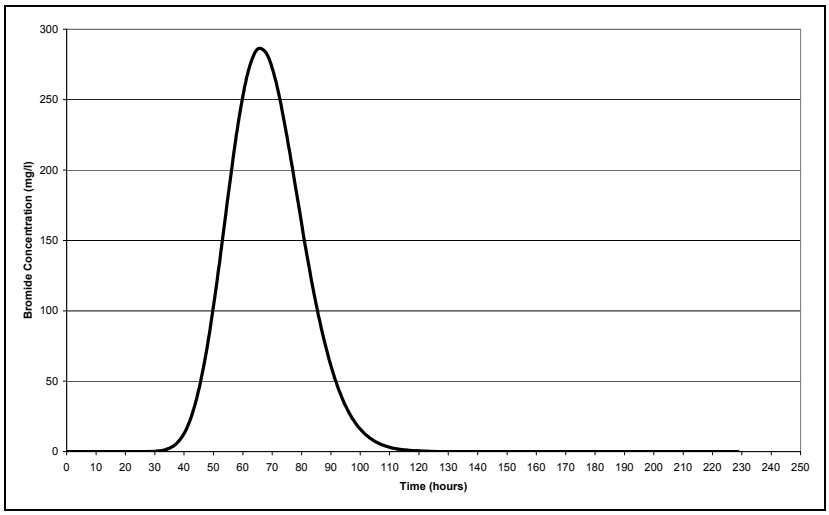


Figure 5.1: Predicted response curve from "CSTRs-in-series model" (Appendix E).

5.2.1 Hydraulics of the DPBR and SSRU2

It is evident from the actual tracer response curve of the DPBR that some short-circuiting occurs within the DPBR (Figure 5.2) as the bromide values were detected in the effluent within an hour after tracer injection. The peak of bromide recorded (61 h after tracer injection), coincided very well with the theoretical retention time of 68 h There was however a lag period involved with residual bromide concentrations being detected well after the theoretical response curve (122-388 h after tracer injection) indicating that there are probably hydraulically limiting areas within the DPBR where flow movement is slow.

The tracer response curve observed for SSRU2 (which has been operational since 1996) indicates that hydraulically limiting areas exist within this bioreactor leading to a prolonged bromide response curve. The bromide peak was only detected 124 h after tracer injection, indicating that SSRU2 is operating at almost double the theoretical retention time (Figure 5.2).

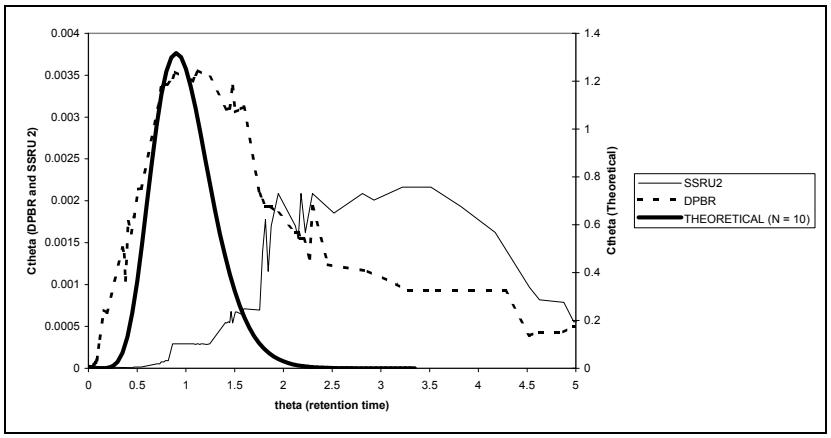


Figure 5.2: Normalised tracer concentration in the effluent (C/C_o) versus normalised time (t/T) of the theoretical response curve for the DPBR and SSRU2.

When comparing the measured bromide concentrations with that of the theoretical response curve, the measured bromide concentrations were significantly lower than the predicted bromide concentrations from the ideal response curve (Figures 5.3). This could be attributed to bromide being absorbed onto the carbon in the bioreactors.

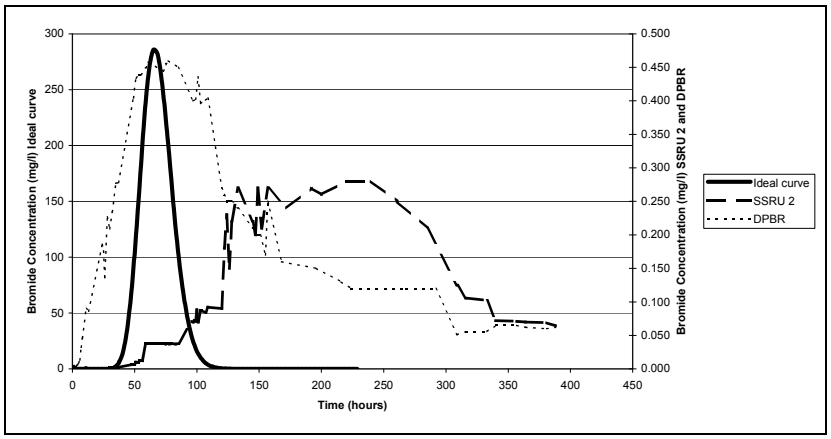


Figure 5.3: Expected bromide concentrations from the predicted response curve versus the actual bromide concentrations measured for the DPBR and SSRU2.

5.3 DISCUSSION AND CONCLUSION

Although there might be hydraulic limitations in the DPBR, the sulfate reduction activity in the DPBR was much higher than that of SSRU2. The causes for deviation from the predicted ideal hydraulic performance are most probably related to quality assurance issues in the packing of the carbon layers in the DPBR. The poor hydraulic performance of the SSRU2 has previously been assessed and ascribed to the truncated inverted pyramid design. Thus the lower sulfate reduction observed in SSRU2 may be attributed to the hydraulic limitations associated with the inverted truncated pyramid geometry of SSRU2 (Fourie, 2002).

CHAPTER 6
GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

6.1 GENERAL DISCUSSION

6.1.1 Long term performance of sulfate reducing units at VCC

The performance of the VCC Passive Treatment plant since start-up in 1996 to March 2005 was previously discussed in detail (Coetser *et al.*, 2005; Coetser and Heath, 2005). None of the standard SRUs achieved the target sulfate load removed of 60 g/m^3 carbon/day. It was previously concluded that the most important parameter restricting sulfate reduction was the prevailing oxic conditions in the bulk of the units. In the presence of significant microbial activity, oxygen present in the system would have been consumed during the biodegradation of organic matter. System malfunction was therefore ascribed to the lack of carbon biodegradation and thus the unavailability of readily biodegradable carbon to drive sulfate reduction.

Table 6.1 summarises the average sulfate concentration removed and the average sulfate load removed of the different units. The DPBR was the only SRU achieving the target sulfate load removed of 60 g/m^3 carbon /d. Although the DPBR was supplemented with molasses, supplementation only contributed an average COD concentration of 71.0 mg/ℓ in the influent to the DPBR while the average concentration of sulfate reduced was 474 mg/ℓ. Molasses could therefore not be the only carbon source responsible for driving sulfate reduction and molasses was probably responsible for the enhancement of ligno-cellulose degradation, which sustained the reduction of sulfate.

Although the DPBR achieved the target sulfate load removed of 60 g/m^3 carbon /d, the effluent sulfate concentrations were still high – averaging 1089 mg/ℓ. It must be kept in mind that the optimum conditions for sulfate reduction did not exist for the DPBR and that sulfate reduction would be higher if the right operating conditions were selected through kinetic column studies. Also the Integrated Managed Passive (IMPI) treatment system consists of more than one sulfate reducing unit and that more SRUs could be incorporated into the system to achieve the discharge water quality objectives.

The supplementation of molasses to SSRU2 and SSRU4 led to increased sulfate loads removed with SSRU2 averaging 45.2 g/m^3 carbon /d and SSRU4 40.2 g/m^3 carbon /d.

Table 6.1: Comparison of average sulfate load removed and sulfate concentration removed of the DPBR and the SRUs.

Unit	Period	Sulfate concentration reduced (mg/ℓ)	Sulfate load reduced (g/m^3 carbon/d)
SSRU1 – Standard	17/10/2002-09/03/2006	71.4	22.3
SSRU2 – Standard	17/10/2004-31/03/2003	Added	6.3
SSRU2 – Molasses	01/04/2003-09/03/2006	252	45.2
DPBR – Standard	17/10/2004-31/03/2005	806	68.6
DPBR – Molasses	01/04/2005-09/03/2006	420	76.5
SSRU4 – Standard	17/10/2004-31/10/2005	12.9	12.4
SSRU4 – Molasses	01/11/2005-11/10/2005	195	40.2
SSRU4 – No molasses	12/10/2005-09/03/2006	164	46.5
LSRU1 – Standard	17/10/2002-09/03/2006	60.8	7.3
LSRU2 – Standard	17/10/2002-09/03/2006	108	11.2

One of the units in the IMPI treatment system, which is still undergoing refinements, is the sulfide oxidation bioreactor (SOBR), oxidising hydrogen sulfide to elemental sulfur. PHD is optimistic about the full-scale implementation of the SOBR after refinements have been finalised by Rhodes University (ongoing WRC funded research). Other potential processes for the removal of sulfides from the system exist including zero-valent iron reactors operated as a passive reactor and electrochemical reactors using iron as a sacrificial electrode in an active reactor.

6.1.2 Hydraulic performance of the sulfate reducing units

Sodium bromide was successfully used as a tracer to generate tracer response curves. It is evident from the tracer response curve of the DPBR that some short-circuiting occurs within the DPBR. However, the bromide peak recorded 61 h after tracer injection, coincided very well with the theoretical retention time of 68 h. A lag period was however involved with residual bromide concentrations being detected well after the theoretical response curve.

The tracer response curve observed for SSRU2, which has been operational since 1996 indicated that hydraulically limiting areas exist within this bioreactor leading to a prolonged bromide response curve.

6.1.3 Three dimensional characterisation of SSRU1

The SRU sampling operation performed in October 2006 was executed successfully and, with the exception of the sawdust layers, it proved possible to identify all the other packing layers from the construction plans. Both water samples and a reactor core sample were recovered. Based on analysis of these, it is possible to draw provisional conclusions relating to the function and possibly also the failure of the conventional SRU packed bed reactor. These observations are considerably strengthened when compared to the studies of DPBR studies undertaken previously as controlled laboratory studies at PHD's carbon facility (Clarke, 2006). Figure 6.1 summarises the trend from top (influent) to bottom (effluent) of the more important parameters evaluated of SSRU1.

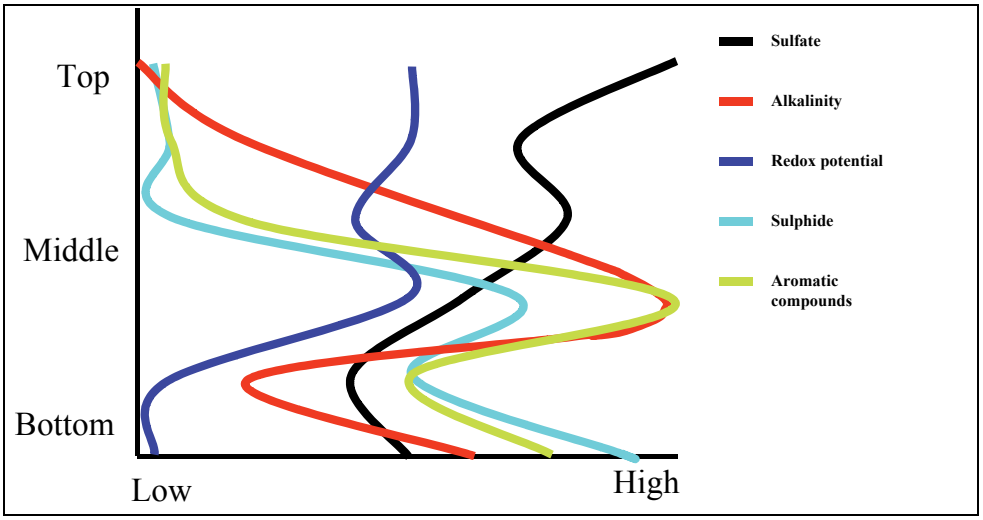


Figure 6.1: Summary of the profile of variables in SSRU1.

Previous attempts to characterise the sulfate reducing units (SRUs), in terms of the liquid fraction, at VCC proved to be very difficult (Cloete, 1998). One of the major drawbacks from characterising the units is the creation of short-circuits within the units after sampling. Only liquid samples were drawn during the study performed in 1998 and no detailed chemical analyses were performed on solid carbon material (Cloete, 1998). Plastic pipes were forced through the carbon and were later sealed with cement. These studies, did however, find that oxic conditions prevailed in the bulk of the units. The presence of oxygen in the units indicated the lack of significant microbial activity, since the latter would have consumed the oxygen during biodegradation of organic matter. From this it was concluded that the biodegradation of the carbon sources in the systems was the rate limiting step.

Hydraulic modelling performed on the VCC SRUs configuration indicated that hydraulic short-circuiting is occurring within the units and that only the centre core of the units are hydraulically active (Fourie, 2002). Fourie (2002) indicated that some sulfate reduction should occur within the centre columns sampled, however, when the overall performance of SSRU1 is evaluated, the effluent sulfate concentrations exceeded the influent sulfate concentrations.

While it was anticipated that a substantial portion of the lignocellulose complex of the woodchip fraction would remain present in the system, even after 9 years of reactor operation, it was surprising to note that even the soluble wood extractives fraction still remained as a substantial component of the woodchip sample. Together with the high residual levels of pectin observed, this indicates extremely poor utilization of the substrate and lignocellulose degradation. These observations are largely supported by the SEM study. It should also be noted that wood particle size plays a role, and that sawdust was more degraded than woodchips over the course of reactor operation.

The SEM results of the woodchip samples did, however, show some differentiation between the Top, Middle and Bottom layers, which was also generally supported by the water sample analyses. It was apparent that the upper layer was more degraded than the woodchip layers lower down in the reactor. Consumption of aromatic compounds, TOC and VFA was also apparently higher in the upper layer. Elevated reducing sugars in this zone also suggested enhanced breakdown of the lignocellulose complex here.

The above results are in agreement with the Degrading Packed Bed Reactor (DPBR) hypothesis which argues that high activity in the upper layer is responsible for sustaining the activity of the full column. However when the easily available substrate for sulfate reduction becomes exhausted in this zone, the whole process gradually unwinds. This corresponds with the trend that emerged and accounts, in part, for the poor performance observed.

This phylogenetic study performed on SSRU1 at VCC provides a very interesting observation of the presence of many of the bacterial physiological types that have also been associated with the successful performance of the DPBRs. Studies, undertaken under controlled laboratory conditions on the DPBRs, have provided a structured outline of the microbial processes occurring in these systems (Clarke, 2006). A more detailed comparison of the bacterial species found during this study and the study performed by Clarke (2006) should be undertaken when the Clarke (2006) work is published.

The observation of comparability of the two results provides a very useful indication that the failure of the SRU system was not due to the lack of appropriate biocatalytic genetic potential. Most of the

necessary forms of bacteria found in the laboratory studies performed on DPBRs were present in SSRU1. However, a readily supply of biodegradable carbon is required to drive the process over the depth of the SRU and enhance lignocellulose degradation.

The results reported here provide an important supportive contribution to the operational hypothesis on which the DPBR reactor design concept is based.

Specialised studies performed on the solid carbon samples are very expensive and not all of the analyses performed proved to be valuable in evaluating the performance of the SRUs. Accurate oxygen determinations also proved to be difficult to measure under anaerobic conditions.

6.2 CONCLUSIONS

6.2.1 Long term performance of sulfate reducing units at VCC

During the operation of a DPBR, readily available carbon or kick-start carbon is fed to the system. This conditions the water by initiating anaerobic metabolic processes removing oxygen from the system and establishing the right redox potentials. Sulfate reduction starts adding sulfides and alkalinity to the system. The presence of high concentrations of sulfides and alkalinity prevents the re-polymerisation of complex carbon such as lignocellulose thereby enhancing the degradation thereof.

The commissioned DPBR has been operating for a period of 3 years and 5 months. Thus far, it has shown a constant and high sulfate reduction activity compared to the standard SRUs. The average sulfate load removed from the DPBR was 76.5 g/m^3 carbon/d. The DPBR was however not packed in accordance with the implementation of the IMPI Process where kinetic column studies would normally be undertaken first with the results then being used to optimise the process according to site-specific parameters.

Molasses supplementation to SSRU2 and SSRU4 has led to an increase in sulfate reduction and sulfide production. The sulfate load removed for SSRU2 increased from 6.3 g/m^3 carbon/d (17/10/2004-31/03/2005) to 45.2 g/m^3 carbon/d after molasses supplementation (01/04/2005-09/03/2006). This represents a 316% improvement in sulfate load removal. The sulfate load removed in SSRU4 increased from 12.4 g/m^3 carbon/d (17/10/2004-31/03/2005) to 40.2 g/m^3 carbon/d after molasses supplementation. This represents an improvement of 324% in sulfate load reduced.

Enhanced sulfate reduction was maintained for a period of 3 years in SSRU2 and for a period of 28 months in SSRU4. The DPBR was successfully maintaining high sulfate loads removed for a period of 3 years and 5 months. The enhancement strategies were therefore implemented with success at the VCC Passive Treatment Pilot Plant.

6.2.2 Hydraulic performance of the sulfate reducing units

The following conclusions can be made from the tracer studies:

- Sodium Bromide was successfully used as tracer to generate tracer response curves.
- With regards to the DPBR:
    - Some short-circuiting occurred within the bioreactor

    - Hydraulically dead spaces occur leading to prolonged retention of the water in the DPBR
    - The bromide peak corresponded well with the theoretical retention time of 68 h.
    - Hydraulic configuration seems to be close to optimum as can be seen by the high sulfate reduction values
- With regards to SSRU2:
    - Hydraulically dead spaces occur leading to prolonged retention of the water in the SSRU2 unit.

The hydraulic configuration used in the DPBR has enabled an optimum diffusion of mine water throughout the reactors as can be seen by the high sulfate reduction values.

6.2.3 Three dimensional characterisation of SSRU1

The following conclusions were made:

- Even after 9 years of operation, the soluble wood extractives fraction still remained as a substantial component of the woodchip sample.
- Substrate utilisation was poor.
- Lignocellulose degradation was poor.

6.3 RECOMMENDATIONS

6.3.1 Long term performance of sulfate reducing units at VCC

It is recommended that the DPBR and the SSRUs operating using the enhancement strategies are monitored further in order to determine the long-term performance of these units.

The implementation of the IMPI process, as patented after the Innovation Fund project, resulted in the desired result of enhanced sulfate reduction at the VCC pilot plant. When comparing the older VCC sulfate reduction units with the DPBR results it is clear that the new DPBR technology has moved the sulfate reduction process to a higher level. The use of the rapid breakdown kick-start carbon source (molasses) has also indicated that it is effective in increasing sulfate reduction for the older sulfate reduction units that were no longer achieving the required performance. The results of the DPBR and the kick start carbon supplementation at VCC have shown encouraging results that need to be monitored for at least another year.

6.3.2 Hydraulic performance of the sulfate reducing units

It is recommended that the experimental programme as described in this report to perform tracer studies is used in future studies to determine the hydraulic performance of the SRUs.

6.3.3 Three dimensional characterisation of SSRU1

Recommendations for future characterisation programmes include:

- The incorporating of dedicated sampling columns during the construction phase of the SRUs, which can be sealed off completely after sampling to prevent short-circuiting within the unit.

- Incorporation of thin perforated pipes within the SRUs to enable *in-situ* sampling over the depth of the column without actual disturbance of the SRUs.
- Focus on the following analysis as these proved to be the most valuable in assisting with the characterisation of the unit:
    - Water samples – sulfate, sulfide, alkalinity, aromatic compounds, volatile fatty acids and reducing sugars determinations.
    - Solid samples – Soluble pectin, lignocellulose fractions determinations as well as scanning electron microscopy studies.
    - Phylogenetic typing.

CHAPTER 7
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APPENDIX A
HISTORY OF PILOT PLANT OPERATION

The VCC pilot plant has been in operation since 1996, and the different phases of operation are summarised in Table A-1.

Table A-1: Summary of the units at the VCC Pilot Plant showing phases and mode of operation (No operational changes were made during the period 1 April 2004 to 9 March 2006).

Date	Phase	Component	Description
Nov 1996 to Dec 1998	1	SSRU1 SSRU2 SSRU3 SSRU4	Small units running in series with all other units
Nov 1996 to April 2002	1 & 2	LSRU1 LSRU2	Large units operated in single step and in parallel
Nov 1996 to Dec 1998	1	HSRU 1 HSRU 2	Units connected in series to SSRU3 and SSRU4
Aug 1999 to April 2002	2	SSRU1 SSRU4	Units connected in parallel to SSRU2 and SSRU3
Aug 1999 to April 2002	1 & 2	SSRU2 SSRU3	Units connected in series with outflow from SSRU2 feeding SSRU3
Aug 1999 to April 2002	2	HSRU 1 HSRU 2	Units decommissioned and not operational
August 2002	3	SSRU3	Decommissioned and recommissioned as a degrading packed bed reactor (DPBR)
April 2003	2	DPBR &SSRU2	Molasses supplementation into the feed water line
November 2003	2	SSRU4	Molasses supplementation into the feed water line

APPENDIX B
DETERMINING NEUTRALIZATION CAPACITY OF SSRU4 FOR ENHANCED LIGNOCELLULOSE DEGRADATION AND IMPROVED SULFATE REDUCTION AFTER TERMINATION OF MOLASSES SUPPLEMENTATION

B-1 Background

Enhancement strategies were implemented on SSRU1 and SSRU4 by supplementing these reactors with molasses. Effluent COD concentrations were continuously higher than influent COD concentrations. This prompted the question whether it is really necessary to feed molasses on a continuous basis to the reactors or whether enhanced lignocellulose degradation can be maintained if molasses is only dosed at certain intervals.

B-2 Materials and methods

Supplementation of molasses to SSRU4 was terminated on 11/10/2005. Influent and effluent of SSRU4 were sampled and analysed in terms of pH and alkalinity as mg/ℓ $CaCO_3$.

B-3 Results and discussion

Figure B-1 illustrates the influent and effluent alkalinity of SSRU4 after termination of molasses supplementation. Although effluent alkalinity decreased over the experimental period, alkalinity values higher than 50 mg/ℓ were still maintained 4 months after termination of molasses supplementation (Figure B-1). SSRU4 continued to neutralise the effluent pH 4 months after molasses supplementation was terminated (Figure B-2).

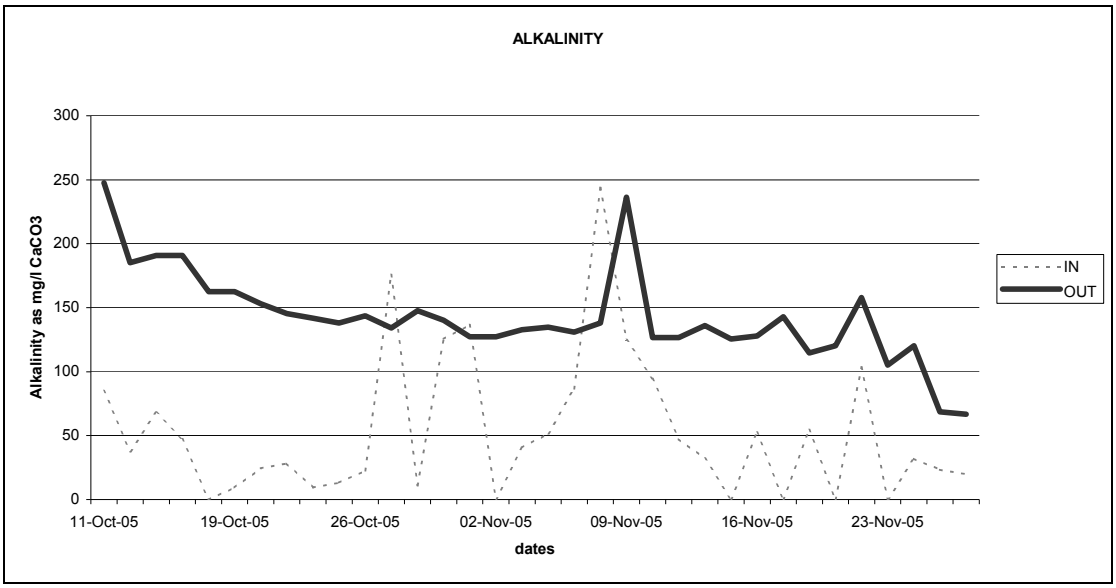

Figure B-1: Influent and effluent alkalinity of SSRU4 after termination of molasses supplementation

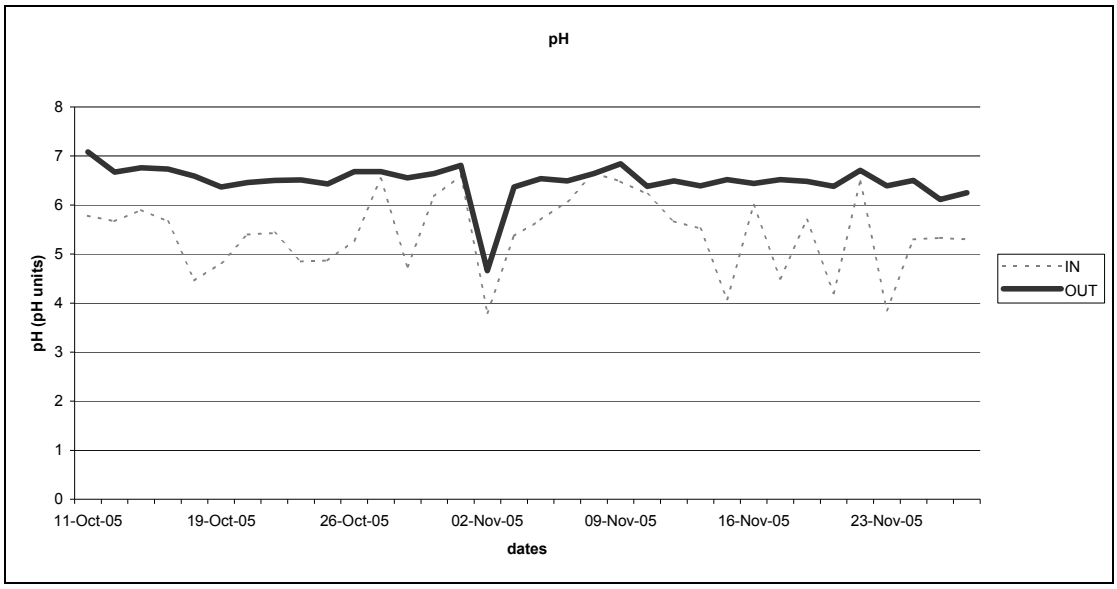

Figure B-2: Influent and effluent pH of SSRU4 after termination of molasses supplementation

Although alkalinity production was still observed and effluent pH's neutralised, it is difficult to evaluate the true performance of SSRU4 after termination of molasses supplementation. Although sulfate reduction was still observed in SSRU4, no alkalinity production and sulfide production were observed when comparing the influent and effluent of the monthly samples taken for chemical analysis.

B-4 Recommendations

It is recommended that SSRU4 is rather monitored using fixed cycles of molasses supplementation followed by no supplementation and extending the periods in between no supplementation. More detailed analysis of influent and effluent should be performed including at least:

- sulfate concentration
- sulfide concentration
- pH
- alkalinity
- redox potential
- COD concentrations

APPENDIX C
METHOD FOR THE THREE DIMENSIONAL CHARACTERISATION OF SSRU1

C-1 CHARACTERISATION OF SSRU1

SSRU1, constructed in 1996 as an inverted truncated pyramid (1.44 m^2 (bottom) x 36 m^2 (top) x 1.8 m (height)), filled with layers of the various organic substrates (Figure C-1; Table C-1) without bulking agent in between the layers, operated as a down flow unit and fed with a 1:1 ratio of South- and West-Adit feed was selected for characterisation.

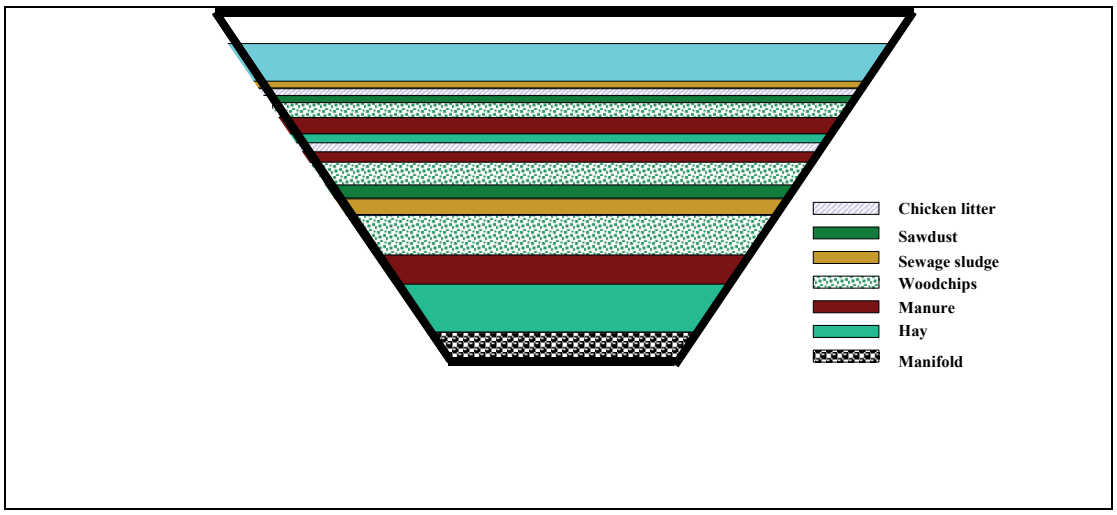

Figure C-1: Layers of carbon placed in SSRU1 (to scale).

Table C-1: Depth of layer, cumulative depth and volume of carbon packed.

Carbon layer	Depth of layer (m)	Cumulative depth (m)	Volume (m^3)
Sewage sludge	0.034	0.034	1.00
Chicken litter	0.035	0.069	1.00
Sawdust	0.036	0.105	1.00
Woodchips	0.078	0.183	2.00
Manure	0.086	0.269	2.00
Hay	0.047	0.326	1.00
Chicken litter	0.050	0.366	1.00
Manure	0.054	0.420	1.00
Woodchips	0.122	0.541	2.00
Sawdust	0.070	0.612	1.00
Sewage sludge	0.080	0.692	1.00
Woodchips	0.206	0.897	2.00
Manure	0.150	1.047	1.00
Hay	0.252	1.299	1.00
Manifold	0.150	1.499	0.29

C-2 SAMPLING OF SSRU1

A temporary wooden deck was constructed over SSRU1 to enable access to the middle of the unit (Figure C-2). Core samples were taken in the middle of the unit as previous studies indicated that

hydraulic short-circuiting occurs within the bioreactors and that only the center part of the units is hydraulically active.

Figure C-2: Temporary wooden deck constructed to enable sampling in centre of SSRU1. (SSRU1 was covered with a bright orange chemical sludge.)

The following equipment was constructed to enable sampling of SSRU1:

- Carbon sampling corer
- Liquid sampling corer
- Liquid sampling device
- Carbon auger

C-2.1 Carbon Sampling Corer

The sides of four 225 mm diameter steel pipes (1800 mm length) were drilled with four rows of holes and the bottom ends sharpened (Ferroland, Kumba Resources) (Figure C-3). The holes in the carbon sampling corers enabled easy access of liquid fraction forming an equilibrium with the surrounding areas. These modified pipes were used as carbon sampling corers.

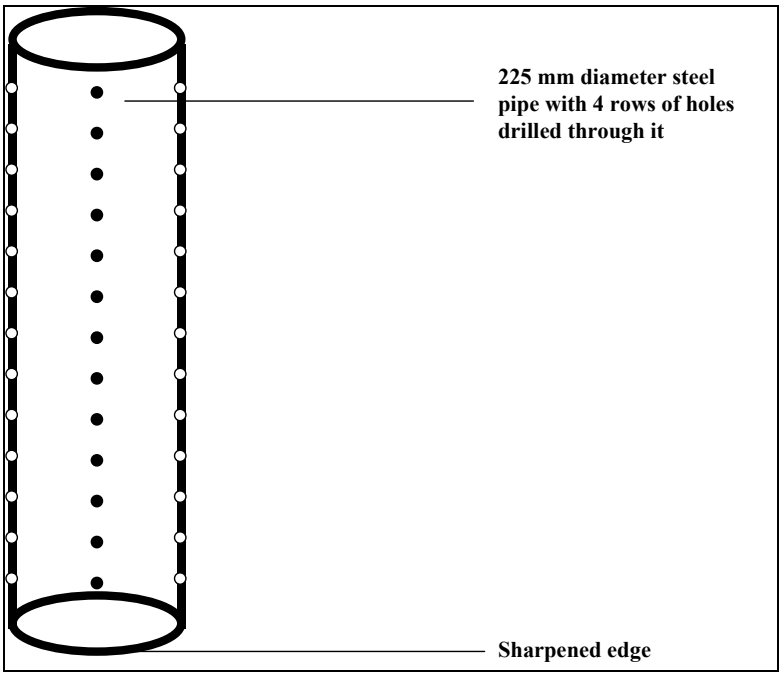

Figure C-3: Schematic illustration of the carbon sampling corer.

C-2.2 Liquid Sample Corer

Four 50 mm diameter perforated steel pipes with lengths of 1800 mm and a sharpened closed end at bottom (constructed by Ferroland, Kumba Resources) were used as liquid sampling corers (Figure C-4). The closed sharpened ends facilitated easier access through the carbon without carbon entering the liquid sample corer.

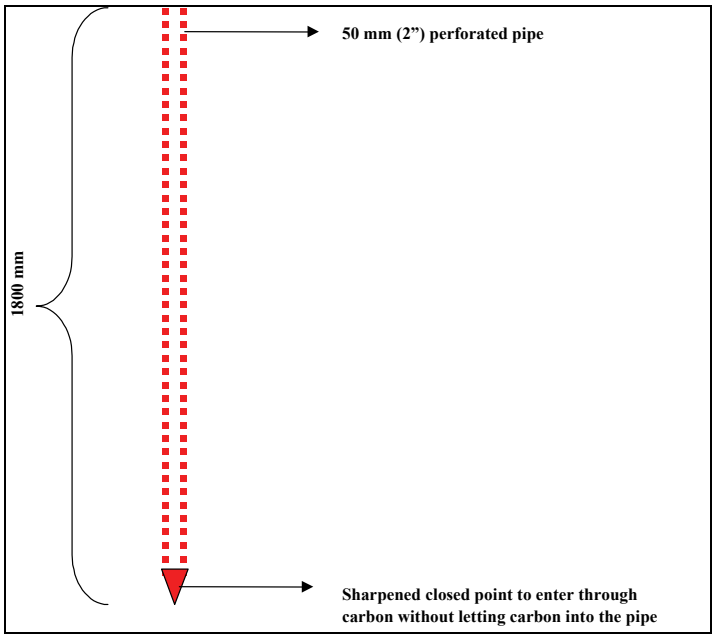

Figure C-4: A schematic representation of the liquid sampling corer.

C-2.3 Liquid Sampling Device

A liquid sampling device was constructed from a 1800 mm length plastic pipe (diameter 188 mm), fitted with a flexible plastic hosepipe and clamped at the top (Figure C-5). The plastic pipe was labelled with measurement points as to determine the depth of sampling.

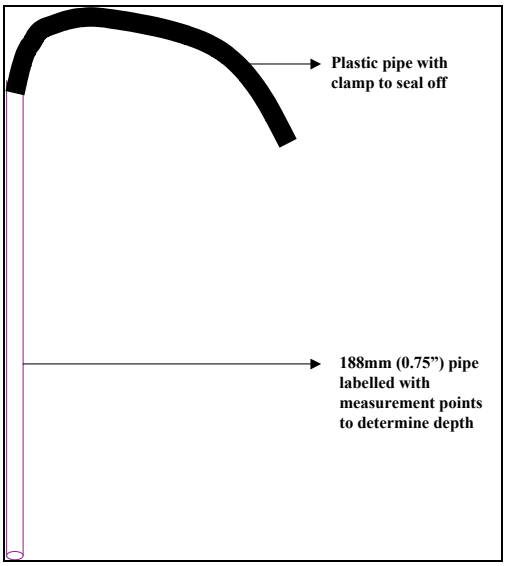

Figure C-5: Schematic representation of the liquid sampling device

C-2.4 Carbon Auger

Ferroland (Kumba Resources) constructed a carbon auger for PHD as illustrated in Figures C-6 and C-7.

Figure C-6: Carbon auger (1)

Figure C-7: Carbon auger (2)

C-2.5 Sampling

Four carbon sampling corers were manually forced through SSRU1 to enter the soil layer below SSRU1. Figure C-8 illustrates a cross section through SSRU1 indicating two corers penetrating into SSRU1. The photograph in Figure C-9 shows the four carbon sampling corers inserted into SSRU1.

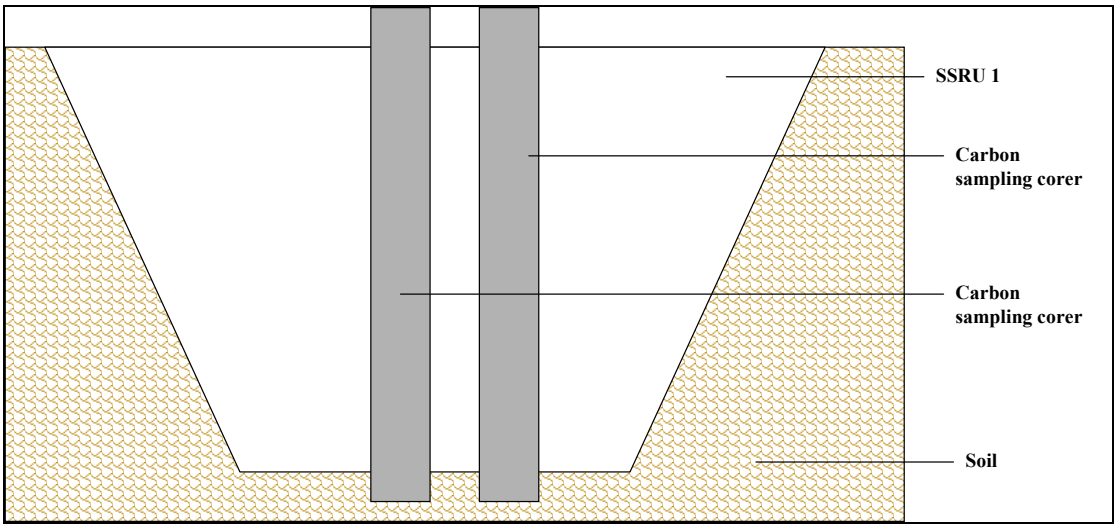

Figure C-8: Schematic diagram illustrating the penetration of the carbon sampling corer through SSRU1 and the underlying soil.

Figure C-9: Placement of the carbon sampling corers (only pipes A and C were successfully sampled).

After inserting the carbon sampling corers, the liquid sample corers were manually forced down through the middle of the carbon sampling corers (Figure C-10). The carbon sampling corers and liquid sample corers were left for a period of 36 h to reach equilibrium with the surrounding areas before actual sampling proceeded.

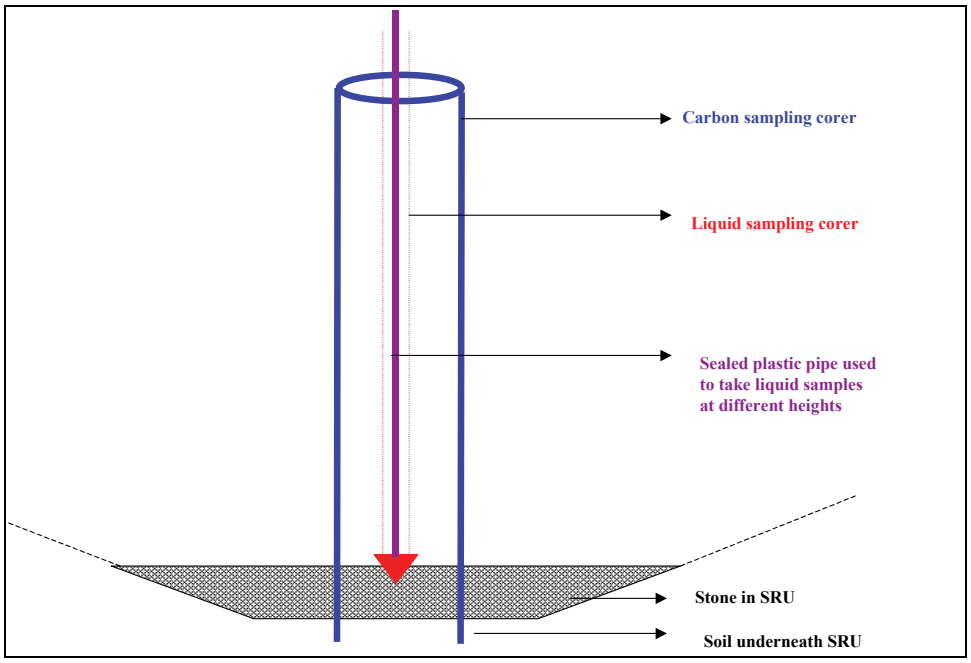

Figure C-10: Schematic representation of the sampling method.

In situ determinations of the following parameters were done at 250 mm intervals by lowering the probe slowly down the liquid sampling core:

- Electrical conductivity
- Total dissolved solids
- Salinity
- Dissolved oxygen
- pH
- Redox potential

Liquid samples were taken for analysis at 250 mm intervals using the liquid sampling device for the top sections. The deeper sampling progressed down the SSRU1, the more difficult it became to extract liquid samples using the liquid sampling device. Lower samples were taken using a small plastic container fixed to a rope. All of the liquid was extracted at each depth before sampling continued to the next section. Liquid samples could only be taken to the bottom of the unit in two of the sampling columns (Column A and Column C) as the other two columns did not provide a water-tight seal and water continued to enter into these columns from the bottom of the unit.

After all of the liquid was extracted from Column A and Column C, the liquid sampling corers were removed from the carbon sampling corers. Liquid samples were refrigerated in a portable refrigerator. The carbon augers were used to extract the solid carbon samples from Column A and Column C and the depths at which samples were taken recorded. Figure C-11 illustrates the carbon auger after carbon has been extracted.

Figure C-11: Carbon auger containing extracted carbon.

Carbon was then removed from the carbon auger keeping the order of the carbon in place as far as physically possible. The carbon was sub-sampled immediately for microbiological analysis.

All of the carbon removed was placed in the correct order, to allow identification of the original layers packed into the reactor before sub-samples were placed into sealed plastic bags and refrigerated. Solid samples were selected based on visual observations. The following solid carbon samples were submitted for analysis as discussed in Chapter 4:

- Sewage sludge/chicken litter mix
- Woodchips – top
- Manure
- Woodchips – middle
- Woodchips – bottom
- Manure/hay mixture
- Hay
- Fresh pine chips (control)

The woodchips samples (top, middle and bottom) and the hay sample were extracted with water to remove the debris from the carbon and are referred to as:

- Woodchips – top extracted
- Woodchips – middle extracted
- Woodchips – bottom extracted
- Hay – extracted

C-3 ANALYTICAL PROGRAMME

A number of physical and chemical analyses were carried out at various laboratories on the liquid (Table C-2) and solid (Table C-3) samples.

Table C-2: Summary of chemical and physical analyses performed on the liquid.

Responsible laboratory	Analysis	Method
PHD	Sulfate, Sulfide, COD, Alkalinity, Iron, Manganese, Aluminium, Calcium, Copper, Lead, Magnesium, Potassium, Sodium, Zinc	APHA,1998
Agricultural Research Council (ARC)	Starch	Glucose concentrations of sample are determined and the values multiplied by the factor 0.912 in order to convert it to % starch
	Water soluble carbohydrates (WSC)	Carbohydrates measured spectrophotometrically against standards (Harris, 1990)
	Total non-structural carbohydrates (TNC)	Digestion of sample with amylase enzymes and carbohydrates measured spectrophotometrically against standards (Faichvey and White, 1983)
Environmental Biotechnology Group (EBRU)	Aromatic compounds	Section C-3.C-5.1
	Total organic carbon (TOC)	Section C-3.C-5.2
	Volatile fatty acids (VFAs)	Section C-3.C-5.3
	Reducing sugars	Section C-3.C-5.4

Table C-3: Summary of chemical analyses performed on the solid samples.

Responsible laboratory	Analysis	Method
ARC	Aqua regia digest	APHA, 1998
	Moisture content	APHA, 1998
	Freeze dried	Vacuum Technologies (Pty(Ltd))
	Dry matter content	Samples are dried overnight at 100ºC to determine dry matter (Harris, 1990)
	Ash content	Ash content is measured after incineration overnight at 550ºC and then for 2 h at 900ºC (Harris, 1990)
	Total Kjeldahl nitrogen/Crude protein content	Crude protein can be estimated as Kjeldahl N x 6.25 using a LECO FP-2000 protein analyser (LECO AFRICA (Pty(Ltd))
	Crude fat	Crude fat is determined through ether extraction (AOAC, 1984)
	Crude Fibre content	% Crude fibre is calculated as follows: $\%\ \text{Crude Fibre} = \frac{\text{RCD} - \text{RCA}}{\text{original sample mass}} \times 100$ where: RCD = residue in crucible after drying RCA = residue in crucible after ashing (AOAC, 1984).
	Lignin, cellulose and hemicellulose	The gravimetric method of Goering and van Soest (1970) is used to determine the concentration of hemicellulose, cellulose and lignin.
	In vitro digestibility	It involves a 48 hour fermentation by rumen micro-organisms in a buffer solution followed by a 48 hour pepsin digestion after acidifying with hydrochloric acid (Tilley and Terry; 1963).
Council for Geoscience	*X-ray diffraction analysis (XRD)	Section C-3.C-7
University of the Witwatersrand	*X-ray fluorescence analysis (XRF) for major oxides (including wt%S and trace metals	Section C-3.C-7
University of Forte Hare	*Mineralogy identification using microscopy	Section C-3.C-7
EBRU	Soluble pectin	Section C-3.C-5.5
	Lignocellulose fractions	Section C-3.C-5.5
	Scanning electron microscopy (SEM) studies	Section C-3.C-5.6
PHD	Water extraction and analysed for metals as listed for liquid samples	APHA, 1998
	Aqua regia digest leachate (Waterlab) analysed for metals as listed for liquid samples	APHA, 1998

*Used for mineralogical and Geochemical analysis

C-3.1 Aromatic Compounds

The release of aromatic compounds was followed by measuring total phenol in the liquid samples with an adaptation of the method by Box (1983), in which Folin-Ciocalteau reagent (Merck), and colour change occurring in the presence of reducing substances, was monitored at 750 nm (Beckman DU® 530 Life Science UV/Vis). The colour development is due to the transfer of electrons at alkaline pH values that reduce the phosphomolybdic/phosphotungstic acid complexes to form chromogens that can be detected spectrophotometrically. Ferulic acid (Sigma), a monomer of lignin, was used to

monitor the appearance of lignin-derived (or wood-derived) aromatic compounds. A standard curve was constructed using ferulic acid.

Prior to analysis, sulfide was removed from solution to prevent interference by acidification to pH <5, then sparged with nitrogen gas for 10 min (Box, 1983). 1.5 mℓ of Sodium Carbonate (200 g/ℓ) and 0.5 mℓ of Folin-Ciocalteau reagent was added to 10 mℓ of liquid sample. The samples were incubated at 20ºC for 60 minutes and then the absorbance was measured at 750 nm. Ferulic acid was used to draw the standard curve.

C-3.2 Total Organic Carbon

Total Organic Carbon was analysed using the Dohrmann Total Organic Carbon Analyzer.

C-3.3 Volatile Fatty Acids

The total VFA were determined by the 5-point titration method.

C-3.4 Reducing Sugars

A modified Somogy-Nelson method was employed for the determination of reducing sugars. This method uses a colorimetric reaction which involves heating the sample in the presence of a copper reagent, an alkaline solution of copper tartrate and cuprous oxide is formed. When combined with arsenomolybdate it reacts to form molybdenum blue which is then measured at 420 nm on a spectrophotometer. A sample was collected into a test tube to which a Nelson solution (Appendix D) was added. After 20 minutes of boiling at 90^{o}C an arsenomolybdate solution (Appendix D) was added to the reaction tube. The reducing sugar absorbencies were determined using a Beckman DU^{R} UV/V is spectrophotometer at 520 nm. The reducing sugar concentrations were then determined from the glucose standard curve.

C-3.5 Soluble, Pectin and Lignocellulose Fractions

The woodchip layers from the Top, Middle and Bottom of the reactor were analysed and fresh woodchips were also sourced as control material for the analysis. The woodchips were washed with distilled water and then they were dried at 40ºC. The samples were then grated into a fine powder, and just over 1 g was used for the analysis. The procedure for the preparation of wood extracts for analysis is shown in Figure C-21.

In order to remove the easily extractable material from the dried wood powder, it was subjected to extraction in ethanol:benzene (1:2, v/v) for 48 h with continual stirring. Residual solids were then filtered through a Buchner funnel and Whatman No 1 filter paper. The residue was washed with benzene followed by ethanol to remove any remaining soluble organic compounds. The treated material was then dried at room temperature, and this material was termed fraction1 and was composed of pectin and lignocellulose.

Depectinated wood powder was produced by treating fraction 1 with potassium acetate (0.12%, w/v) for 24 hours at 60ºC with continual stirring. The residue was filtered as before and allowed to dry at

room temperature. The residue was termed fraction 2, and corresponds to the lignocellulose complex, the core structure of wood tissue.

The pectin contained in each wood sample was determined by subtracting the weight of fraction 2 from the weight of fraction 1.

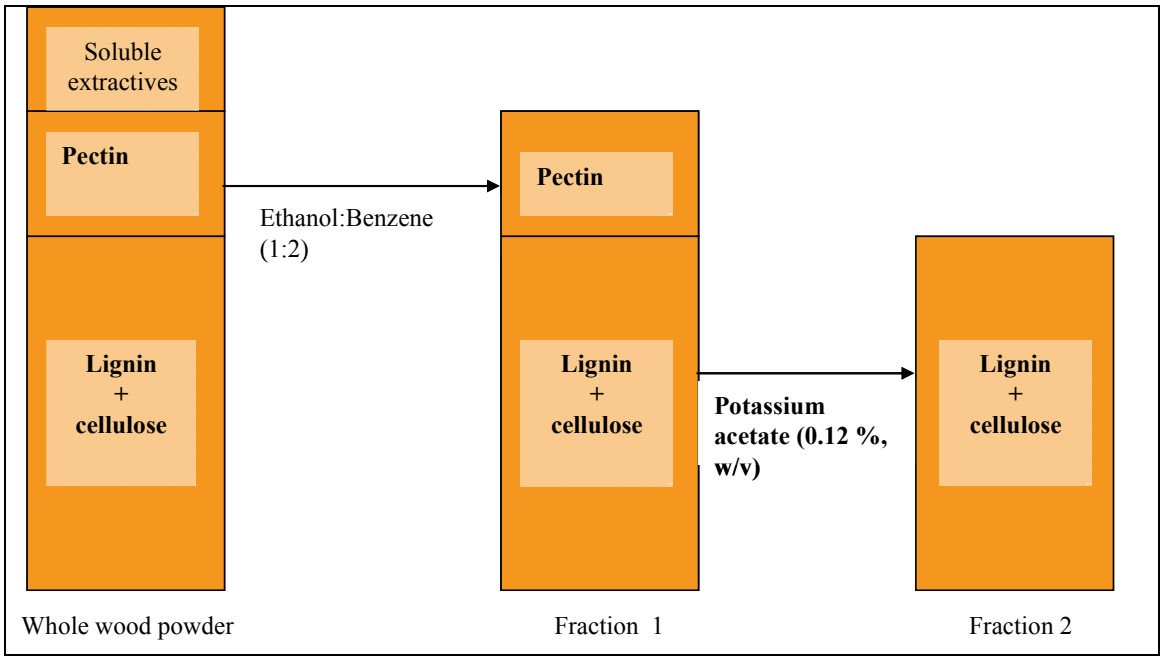

Figure C-12: Procedures for the preparation of wood extracts used in this study. Soluble extractives were removed with ethanol:benzene to give fraction 1, pectin was removed using potassium acetate to give fraction C-

C-3.6 Scanning Electron Microscopy of Woodchip Fractions

Scanning Electron Microscopy (SEM) was carried out on the wood samples (fresh, top, middle and bottom). The samples were prepared for SEM following Cross *et al.*, 2001. The samples were fixed in a C-5 % solution of buffered glutaraldehyde (Appendix D) at 4°C overnight. The buffered glutaraldehyde was poured off and the sample was washed twice with phosphate buffer (Appendix D). Once the wash steps were completed the sample was put through an alcohol dehydration series. The alcohol concentrations used were 30% through to absolute ethanol in a 10% dilution series, each step was left for 10 minutes. The sample was then placed into a critical point dryer apparatus basket where it was covered with absolute ethanol.

Critical point drying prepared the sample without causing any distortion or collapse and avoids these problems by preventing the passage of the liquid/vapour interface through the specimen during the drying process (Cross *et al.*, 2001). This was achieved by raising the temperature and pressure of the liquid filling and surrounding the specimen to above its critical point, at which stage there was no longer an interface between the liquid and vapour phases. The temperature was then maintained at above its critical point and the pressure was slowly reduced, the liquid in the specimen was bled out as a vapour from the pressure vessel (Cross *et al.*, 2001).

The sample was then gold-coated in order to improve the secondary electron emission and to reduce charge build up. The sample was then placed in the Scanning Electron microscope JEOL JSM 840 for viewing.

C-4 MOLECULAR STUDIES (EBRU)

Molecular phylogenetic analysis was undertaken on samples from the different levels within the reactor. Molecular samples were drawn immediately the auger was removed from the sampling pipe and then the solid samples were laid out on the surface representing the depth profile as shown in Table 4.1.

C-4.1 DNA Extraction

DNA extraction and purification was carried out using a method adapted from Bond (Bond *et al*, 2000). Samples (0.5 mℓ) were pelleted by centrifugation, washed once with phosphate buffered saline (PBS) pH 1.2, washed with one part 2 X Buffer A (200 mM Tris pH 8.0, 50 mM ethylenediaminetetreacetic acid disodium salt (EDTA), 200 mM sodium chloride (NaCl), 2 mM sodium citrate, 10 mM calcium chloride ($CaCl_2$) and one part 50% glycerol and then resuspended in 0.5 mℓ 2 X Buffer A. Lysozyme (3 mg/mℓ) was added and the sample was incubated in a 37ºC waterbath for 1 h. Proteinase K (2 mg/mℓ) was added and the sample was incubated in a 50ºC waterbath for 30 minutes. SDS was added to a final concentration of 5%. The samples were then treated by four cycles of freeze-thawing. This entailed being frozen in liquid nitrogen for 1 minute and then placed in a 90ºC waterbath for 3 minutes. 500 µℓ of sample was then added to 500 µℓ of phenol (pH 8), vortexed for 10 seconds and the phases were separated by centrifugation. The upper aqueous phase was removed to a fresh tube and 250 µℓ phenol and 250 µℓ chloroform:isoamyl alcohol (24:1) was added. The sample was vortexed for 10 seconds and the phases were separated by centrifugation. The upper phase was removed to a fresh tube and 500 µℓ chloroform:isoamyl alcohol (24:1) was added. The sample was vortexed for 10 seconds and the phases were separated by centrifugation. The upper phase was transferred to a fresh tube. 50 µℓ of 3 M sodium acetate was added and the 1.5 mℓ tube was filled with 96% rectified ethanol and incubated at –20ºC overnight. The DNA was collected by centrifugation at 4ºC and 13 000 rpm for 30 minutes. The alcohol was poured off and the DNA pellet was left to dry. The DNA was resuspended in 50 µℓ sterile water and stored at –20ºC. The DNA was checked by agarose gel electrophoresis.

C-4.2 Agarose Gel Electrophoresis (Sambrook *et al*., 1989)

The results of the DNA extractions were viewed by using agarose gel electrophoresis. First a 1% agarose gel was prepared, this consisted of 0.5 g of molecular grade agarose and 50 mℓ of 1 x TBE Buffer (Appendix D), this mixture was boiled until the agarose was completely dissolved and then cooled to approximately 50˚C. 50 µℓ of ethidium bromide stock solution (Appendix D) was added and then the agarose gel was poured into a gel mould, a comb was placed into the gel and it was allowed to set. Once set, the gel was placed in a gel tank containing 1 x TBE buffer.

The 10 µℓ DNA extraction samples had 2 µℓ DNA loading buffer (Appendix D) added to them before they were loaded onto the gels. A *λPst1* molecular weight marker (Appendix D) was loaded onto the gel in order to determine the molecular weight of the bands on the gel by drawing a standard curve.

The gels were generally run at 100V of 1 hour and then viewed on a UV lightbox and photographed using a Kodak Digital camera.

C-4.3 Polymerase Chain Reaction

PCR was performed on the DNA extraction samples from the SRU. Each 25 µℓ PCR reaction contained: 2 µℓ of 10 mM GM5F (5' - cct acg gga gcagc ag - 3') primer stock solution (Muyzer *et al.,* 1993; Santegoeds *et al.,* 1998), 2 µℓ of 10 mM 907R (5' - cgc ccg ccg cgc ccc gcg ccc gtc ccg ccg ccc ccg ccc gcc gtc aat tcc ttt gag ttt - 3') primer stock solution (Muyzer *et al.,* 1993; Santegoeds *et al.,* 1998), 1 µℓ 10 mM dNTP stock solution, C-5 µℓ *Taq* DNA polymerase 10 x PCR buffer without $MgCl_2$, 3.5 µℓ *Taq* DNA polymerase 25 mM $MgCl_2$ stock solution, 0.2 µℓ *Taq* DNA polymerase (5 U/µℓ), 1 µℓ DNA (200-500 ng/µℓ chromosomal DNA), 1 µℓ BSA (5 mg/mℓ) and 11.5 µℓ Sigma water.

The PCR reactions were placed in the PCR *Sprint* thermocycler from Hybaid and the Touchdown thermocycle program was run, Table C-4.

Table C-4: Thermocycler programme used for PCR amplification.

Step	Temperature	Time	No. of cycles
Initial Denaturation	95°C	2 minutes	1 cycle
Denaturation	94°C	30 seconds	4 cycles
Annealing	68°C	45 seconds	
Extension	72°C	2 minutes	
Denaturation	94°C	30 seconds	4 cycles
Annealing	66°C	45 seconds	
Extension	72°C	2 minutes	
Denaturation	94°C	30 seconds	4 cycles
Annealing	64°C	45 seconds	
Extension	72°C	2 minutes	
Denaturation	94°C	30 seconds	4 cycles
Annealing	62°C	45 seconds	
Extension	72°C	2 minutes	
Denaturation	94°C	30 seconds	12 cycles
Annealing	60°C	45 seconds	
Extension	72°C	2 minutes	
Final Extension	72°C	5 minutes	1 cycle

The PCR product was analysed on 1% agarose gel containing ethidium bromide and visualised on a UV transiluminator (UVP BioDoc-It™ system) fitted with a digital camera. PCR bands were approximately 586bp (Chapter 4).

C-4.4 Transformation and Cloning

The PCR product was cloned into the pGEM®-T Easy Vector system (Promega, USA) as per manufacturer's instruction and transformed into high efficiency *E. coli* JM109 competent cells.

C-4.5 Preparation of Competent *E. coli* JM109 (Adapted from Sambrook et al., 1989)

Competent *E. coli* cells were prepared by inoculating a 5 mℓ test tube of Luria broth (LB) (Appendix B) with JM109 cells, these were shaken overnight at 37ºC. Four 100 mℓ LB flasks were inoculated with

1.5, 1, 0.7 and 0.3 mℓ of the pre-inoculum and incubated at 37ºC on the orbital shaker for approximately 2 hours. The cultures were allowed to grow until the absorbance of the 1.5 mℓ inoculated flask reached an absorbance of 0.6-0.8 (OD_{600}). These flasks were then placed on ice for 5-10 minutes.

The cultures were centrifuged for 10 minutes at 5 000 rpm using the Beckman JA 14 rotor, all centrifugation steps were performed at 4°C. The supernatant was discarded and the pellet resuspended in 50 mℓ of RF1 (100 mM KCl, 50 mM $MnCl_2$, 30 mM CH_3COOK, 10 mM $CaCl_2$, 15% m/v glycerol, pH 5.8). This was incubated on ice for 20 minutes. The cells were then pelleted by a 10 minute centrifugation step as above. The supernatant was decanted and the four pellets were resuspended in 4 mℓ RF2 (10 mM MOPS, 10 mM KCl, 75 mM $CaCl_2$, 15% m/v glycerol, pH 6.8) and the flask contents were pooled. The cells were aliquoted in 150 µℓ volumes and were stored at –80ºC, until use.

C-4.6 Ligations (pGEM-T Easy Vector System)

PCR products for cloning were ligated into the pGEM-T Easy Vector system. Ligation reactions with a final volume of 10 µℓ were set up as per Table C-5 and then incubated at 4°C overnight.

Table C-5: Ligation Reactions for pGEM-T Easy Vector.

	Standard Reaction	Positive Control	Background Control
2 x Rapid Ligation Buffer	5 µℓ	5 µℓ	5 µℓ
pGEM-T Easy Vector	1 µℓ	1 µℓ	1 µℓ
PCR product	1 µℓ	-	-
Control Insert	-	2 µℓ	-
T4 DNA Ligase 3	1 µℓ	1 µℓ	1 µℓ
dddH_2O	2 µℓ	1 µℓ	3 µℓ

C-4.7 Transformation (Sambrook et al., 1989)

The ligations were incubated at 4°C overnight and then 5 µℓ of each ligation reaction was added to 50 µℓ of thawed competent *E. coli* cells, the tubes were mixed and left on ice for 20 minutes. The cells were heat-shocked at 42ºC for 45 seconds and then cooled on ice for 5 minutes. 1 mℓ of SOC medium (SIGMA) was added and the tubes were placed at 37ºC for 1 hour to allow the cells to recover.

200 µℓ of the cells were plated onto LB plates containing 100 µg/mℓ Ampicillin and spread with 4 µℓ IPTG stock solution (200 mg/mℓ) and 40 µℓ X-GAL stock solution (100 mg/mℓ). The plates were incubated at 37ºC overnight.

Colonies containing an insert would have appeared white and those that just had the plasmid appeared blue. The white colonies were picked off the standard reaction plates into 5 mℓ LB test tubes using sterile toothpicks and the cultures were grown overnight at 37ºC.

Confirmation of correct size insertion was done by performing a plasmid extraction on the overnight cultures and then a plasmid digest. The restriction endonuclease digestion products were resolved on a 1% agarose gel.

C-4.8 Plasmid extraction

The plasmids were extracted from the cells using the QIAprep Spin Miniprep Kit from QIAGEN®. The white colonies tooth-picked into 5 mℓ LB test tubes were grown at 37ºC overnight. 1 mℓ of each culture was placed into a sterile Eppendorf tube, the cells were pelleted by centrifuging them at 13 000 rpm for 1 minute. The supernatant was discarded and another 1 mℓ volume of culture was added, the centrifugation step was repeated.

The pelleted cells were resuspended in 250 µℓ of Buffer P1. 250 µℓ of Buffer P2 was added and the tube was inverted 5 times in order to mix. 350 µℓ of Buffer N3 was added and the tube was inverted immediately but gently 5 times. The sample was centrifuged at 13 000 rpm for 10 minutes, the supernatant was then applied to a QIAprep spin column. The sample was centrifuged for 1 minute and the flow-through was discarded. The QIAprep spin column was washed using 750 µℓ of Buffer PE and then centrifuged at 13 000 rpm for 1 minute. The flow-through was discarded and the samples were centrifuged for an additional 1 minute in order to remove any residual wash buffer.

The QIAprep column was placed into a sterile 1.5 mℓ Eppendorf tube and 50 µℓ of sterile water was added to the centre of the column in order to elute the DNA. The samples were left to stand for 1 minute and then centrifuged at 13 000 rpm for 1 minute. The DNA was placed at 4°C for short-term storage and at –20°C for long-term storage.

C-4.9 EcoRI Digest

The plasmid extraction had to be digested with *Eco*RI in order to check whether the insert was correct. The *Eco*RI enzyme cuts on either side of the inserted fragments and in some cases in the centre of the fragment. The correct result would be a 586 bp fragment or two smaller fragments whose combined size added up to 586 bp.

10 µℓ of each QIAprep plasmid extraction was used for the digest. The digest consisted of 0.2 µℓ of *Eco*RI (10U/µℓ), 2 µℓ Buffer H, 10 µℓ plasmid and 7.8 µℓ of $dddH_2O$. The plasmid digest was mixed and placed at 37°C for 3 hours. The restriction endonuclease digestion products were resolved on a 1% agarose gel.

The *Eco*RI enzyme cuts on either side of the inserted fragments and in some cases in the centre of the fragment. The correct result would be a 586 bp fragment or two smaller fragments whose combined size added up to 586 bp. If the clones contained the correct size fragment, they were used for DNA sequencing.

C-4.10 Thermocycling using the BigDye™ Terminator v3.0 Ready Reaction Cycle Sequencing Kit (Applied Biosystems)

The Thermocycling reaction sample consisted of 200-500 ng of plasmid DNA, 1 µℓ 10 µM SP6F primer stock solution, 4 µℓ BigDye, 2 µℓ 5 x dilution buffer and distilled water (to volume of 20 µℓ). The reaction samples were placed in the GeneAmp® PCR system 9700 thermocycler and the cycle sequencing method recommended in the manual was used.

C-4.11 Purification of the Extension Products by alcohol precipitation

The samples were removed from the thermocycler and 5 µℓ of 125 mM EDTA and 60 µℓ 100% ethanol was added. The samples were incubated at room temperature for 15 minutes and then centrifuged at 13000 rpm for 30 minutes. The supernatant was removed and 100 µℓ of ice cold 70% ethanol was added, the sample was centrifuged at 13000 rpm for 15 minutes. The supernatant was removed and the pellet was dried using a 5 minute denaturation program on the thermocycler.

Each purified DNA sample was resuspended in 10 µℓ of deionised formamide, the samples were vortexed and spun down. The samples were then heated to 95°C for 2 minutes in order to denature them and then chilled on ice until ready to use.

Procedures outlined in the ABI PRISM 310 Genetic Analyser User's Manual (P/N 903565) were followed for the sequencing process.

C-4.12 Analysis of the Sequencing Results

Chromatograms were generated by the ABI PRISM® Genetic Analyser Data Collection system C-0.1 by Applied Biosystems. These chromatograms were converted into text format using Chromas software and then put into the NCBI BLAST database. BLAST or Basic Local Alignment Search Tool is a set of similarity search programs designed to explore all of the available sequence databases (Altschul *et al.*, 1997). The percentage similarity to an identified species was recorded, also the length of the match and the E value, which is important for determining the accuracy of the result.

ClustalX was then used to align all the sequences. The alignment was entered into the Phylip programme and a phylogenetic tree was produced showing the relationship of the isolated clones to known organisms on the blast database.

C-5 MINERALOGICAL AND GEOCHEMICAL ANALYTICAL METHODOLOGY

Double polished thin sections of the different carbon were prepared at the school of Geosciences, University of the Witwatersrand, Johannesburg, which were used for microscopic study. Microscopy was carried in the Department of Geology, University of Fort Hare, Alice.

X-ray diffraction analysis (XRD) was carried out at the Council for Geoscience. It provides mineral components and concentrations in wt%, but is semiquantitative. It has a detection limit of 1wt%. X-ray fluorescence analysis (XRF) was carried out at the School of Geosciences, University of the Witwatersrand, Johannesburg. The XRF analysis reports the 10 ordinary major oxides in wt% and

trace elements in ppm. The XRF analysis is a very accurate analytical procedure. Although sulfur concentration was also reported, no standard error was reported, as sulfur determination using XRF analysis is still in the testing stage.

APPENDIX D
REAGENT USED FOR PHYLOGENETIC STUDIES

Phosphate buffer

Solution A 35.8 g/ℓ $Na_2HPO_4.2H_2O$
Solution B 13.6 g/ℓ KH_2PO_4
To achieve a molarity of 0.1 and a pH 7.3 these solutions are mixed in the ratio 4 parts Solution A : 1 part Solution B.

Buffered glutaraldehyde

10 mℓ 25% ultrastructure grade glutaraldehyde
90 mℓ Phosphate buffer
This solution is stored in a dark bottle at 4°C.

10% SDS (100 mℓ)

10 g SDS
100 mℓ $dddH_2O$
Warm to 65°C to allow SDS to dissolve.

Tris-buffered Phenol (pH 8)

Melt 100 g of phenol at 68ºC and then add an equal volume of 0.5 M Tris-HCl (pH 8) at room temperature. Stir on magnetic stirrer for 15 minutes. When the two phases have separated, aspirate the upper aqueous phase. Add an equal volume of 0.1 M Tris-HCl (pH 8) to the phenol and stir. Extract the upper aqueous phase. Repeat the extraction procedure until the pH of the phenol is greater than 7.8. Add 0.1 x volume of 0.1 M Tris-HCl (pH 8) containing 200 µl of β-mercaptoethanol and store the phenol at 4ºC in dark bottle. The phenol should be made in a fume hood and pH checked using pH paper.

Chloroform:Isoamyl alcohol (24:1) (100 ml)

96 mℓ chloroform
4 mℓ isoamyl alcohol

10 x TBE Buffer (1 l)

107,8 g Tris base
55 g Boric Acid
7.44 g di-sodium EDTA
Make up to 800 mℓ with $dddH_2O$ and pH to 8.3 with boric acid. Make up to 1 ℓ and autoclave. Dilute 1 in 10 for 1 x TBE.

Ethidium Bromide (500 mg/ml)

Dissolve 0.5 g of ethidium bromide powder in 1 mℓ of $dddH_2O$, store in a dark bottle at room temperature.

DNA Loading Buffer(6 x)

0.25%	Bromophenol blue
0.25%	Xylene cyanol
30%	Glycerol

***λPst1* Molecular Weight Marker**

Digest 200 µℓ λDNA (0.25 ug/ul) with 24 µℓ of 10 x buffer H and 10 µℓ of *Pst1* enzyme for 3 hours at 37º C. Add 550 µl TE buffer (pH 8) and 150 µℓ 6 x loading buffer. Aliquot 100 µℓ into eppendorfs and store at –20°C.

10 mM dNTP`S

30 µℓ of each nucleotide (dATP, dTTP, dCTP & dGTP) is added into an eppendorf. 180µℓ of $dddH_2O$ is added, bringing the total volume to 300µℓ. Aliquot into eppendorfs (60 µℓ volumes) and stored at –20ºC.

Competent Cells

RF1

100 mM	KCl
50 mM	$MnCl_2$
30 mM	CH_3COOK
10 mM	$CaCl_2$
15%	Glycerol

Make up to 1 ℓ with $dddH_2O$ and adjust the pH to 5.8 with Acetic acid.

RF2

10 mM	MOPS (4-Morpholine-propanesulfonic acid)
10 mM	KCl
75 mM	$CaCl_2$
15%	Glycerol

Make up to 200 mℓ with $dddH_2O$ and adjust the pH to 6.8 with KOH.

Luria Broth (LB)

5 g	Tryptone
2.5 g	Yeast Extract
2.5 g	NaCl

Make up to 500 mℓ with $dddH_2O$. Adjust the pH to 7 with NaOH and sterilize by autoclaving.

LB-Ampicillin Agar Plates

5 g	Tryptone
2.5 g	Yeast Extract
2.5 g	NaCl
7.5 g	Agar

Make up to 500 mℓ with $dddH_2O$. Adjust the pH to 7 with NaOH and sterilize by autoclaving, allow to cool to 60° and add 500 µℓ of 100 mg/mℓ Ampicillin stock.

SOC medium

2 g	Tryptone
0.5 g	Yeast extract
0.05 g	NaCl
10 mℓ	250 mM KCl

Dissolve in 85 mℓl $dddH_2O$, adjust pH to 7. Make volume up to 95 mℓ with $dddH_2O$, sterilize by autoclaving and add 1 mℓ filter sterilized 2M $MgCl_2$ and 1 mℓ filter sterilized 2M glucose stock solution. Aliquot in 1 mℓ volumes and store at –20°C.

IPTG Stock solution (200 mg/mℓ)

0.2 g	IPTG
1 mℓ	$dddH_2O$

Store at –20°C, use 4 µl per agar plate.

X-Gal (100 mg/mℓ)

0.1 g	X-Gal
1 mℓ	dimethylformamide

Store at –20°C, in a dark bottle, use 40 µℓ per agar plate.

Reducing Sugars Determination

Cu^{2+} Reagent

Reagent	**Quantity for 1 ℓ**
$CuSO_4.5H_2O$	4 g
Na_2CO_3	24 g
$C_4H_4KNaO_6.4H_2O$	16 g
Na_2SO_4	180 g
dH_2O	1000 ml

Arsenomolybdate Reagent

Reagent 1

Reagent	**Quantity for 1 ℓ**
$(NH_4)_6Mo_7O_{24}4H_2O$	50 g
dH_2O	900 mℓ
Conc H_2SO_4	42 mℓ

Reagent 2

Reagent	**Quantity for 1 ℓ**
$Na_2HasO_4.7H_2O$	6 g
dH_2O	50 mℓ

Mix reagents 1 and 2 and incubate at 37°C for 24 hours and store in a brown bottle.

APPENDIX E
HYDRAULIC PERFORMANCE OF THE SULFATE REDUCING UNITS (SRUS) AT THE VRYHEID CORONATION PASSIVE TREATMENT PLANT

E-1 BACKGROUND

Previous work carried out on the SRUs (constructed as inverted truncated pyramids) by the Pollution Research Group at the University of Natal (UN) (1999) indicated that there may well be a problem with the hydraulic efficiency of the reactors, particularly as regards to unutilised carbon volume in the reactor. Figure E-3 shows the contours of velocity predicted in the UN report. Their model assumed a quarter of a truncated pyramid and was thus a three-dimensional model with two axes of symmetry. The University of the Witwatersrand modelled water movement through the same SRUs using the study conducted by UN as a basis for comparison. Once again, a 2-dimensional, axis symmetric finite element model was used. Purely down flow conditions were simulated. Figure E-1 shows the contours of predicted vertical velocity from the 2-D analysis, and as can be seen, they were similar to those obtained by the UN authors. Of particular note was the observation of differences in vertical velocity of two orders of magnitude between the axis of the reactor and the outer zone (*i.e.* toward the upper corner of the sloped base). Quite clearly, there is indeed a large volume of substrate that has a limited (if any) flow through it

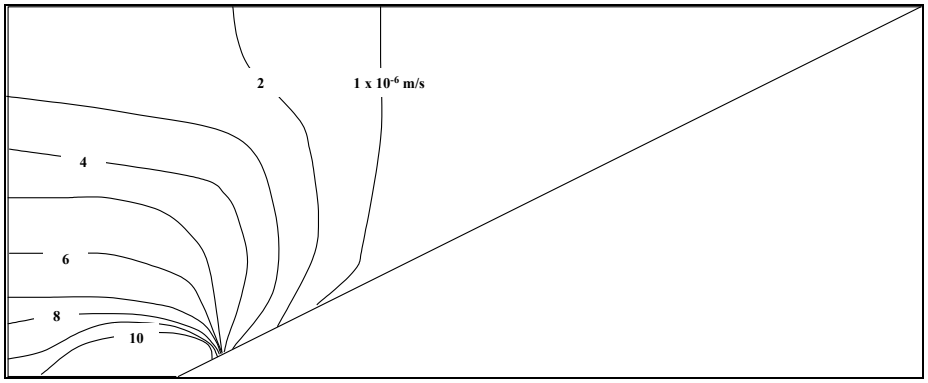

Figure E-1: Contours of vertical flow velocity predicted at VCC SRU by computational fluid dynamics modelling of the large scale down flow reactor (University of Natal, 1999).

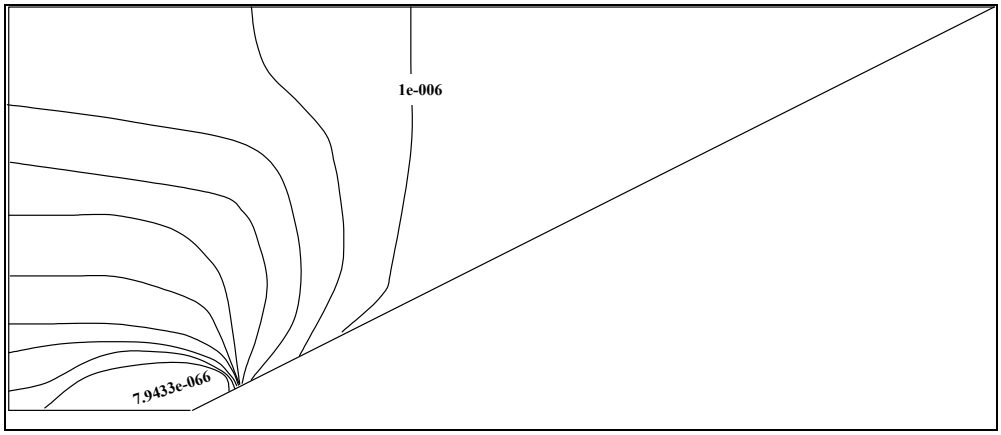

Figure E-2: Contours of vertical flow velocity predicted at VCC SRU by finite element simulation of down flow reactor by the University of the Witwatersrand (Fourie, 2002).

In order to overcome the hydraulic limitations of the original SRUs built as inverted truncated pyramids and based on findings from the optimisation of the geometry performed by Fourie (2002), one of the SSRU's was decommissioned and a Degrading Packed Bed Reactor (DPBR) based on the patented Integrated Managed Passive (IMPI) Treatment Technology was constructed. The DPBR was built with a square geometry (the total volume of the DPBR was the same as for the small SRUs).

Whenever non-homogeneous material *e.g.* grass, wood chips are used as packing material, it raises concerns as to whether the flow is homogeneous or whether there is a high degree of preferential flow occurring, accompanied by the development of a significant number of "dead zones" (*i.e.* zones where flow is severely restricted or prevented).

E-2 THEORETICAL FOUNDATION

E-2.1 Residence time distribution (RTD)

The time required for a particle to travel through a reactor is defined as the residence time of the particle (T) (Levenspiel, 1972). Consider a number of particles that all enter a reactor at exactly the same time. Depending on the mixing regime present, the particles may not all follow the same path through the reactor. The different particles may therefore have different residence times. The distribution of residence times of the set of particles is defined as the Residence Time Distribution (RTD) (Levenspiel, 1972). The RTD of a reactor can be determined by measuring the response, in the outflow, to a tracer material injected into the influent stream. The RTD and tracer response curves for ideal hydraulic behaviour of a reactor are easy to predict. In the case of non-ideal behaviour it is not a trivial matter to predict the RTD and response curve and mathematical models are used to simulate the curve (Levenspiel, 1972). The simulated RTD is then fitted to the measured RTD by adjusting certain variables. These variables may then be used to compare and assess the hydraulic behaviour of different reactors (Levenspiel, 1972).

E-2.2 Obtaining the RTD from a response measurement

The tracer response curve is obtained by plotting the concentration (C) of the tracer in the effluent stream of the reactor versus time (t) (Levenspiel, 1972). The two most frequently used approaches employed to inject the tracer into the reactor are the pulse input and the step input. The response to a pulse input, where a high concentration of tracer is injected as one pulse, is shown as an example in Figure E-3 (Levenspiel, 1972). A step input is obtained by adding a tracer to the influent stream at a constant rate (Figure E-4). The curve in Figure E-3 in is known as a C-curve while the curve in Figure E-4 is called an F-curve.

E-2.3 Ideal hydraulic behaviour

Two extreme flow regimes may be defined for ideal hydraulic behaviour in a reactor vessel:

- The perfect plug-flow reactor
- The Completely Stirred Tank Reactor (CSTR) (Levenspiel, 1972)

The RTD for each of these reactors for the different tracer injection methods is shown in Figures E-3 and E-4. The behaviour of all “real life” reactors can be classified as lying somewhere between these two extremes.

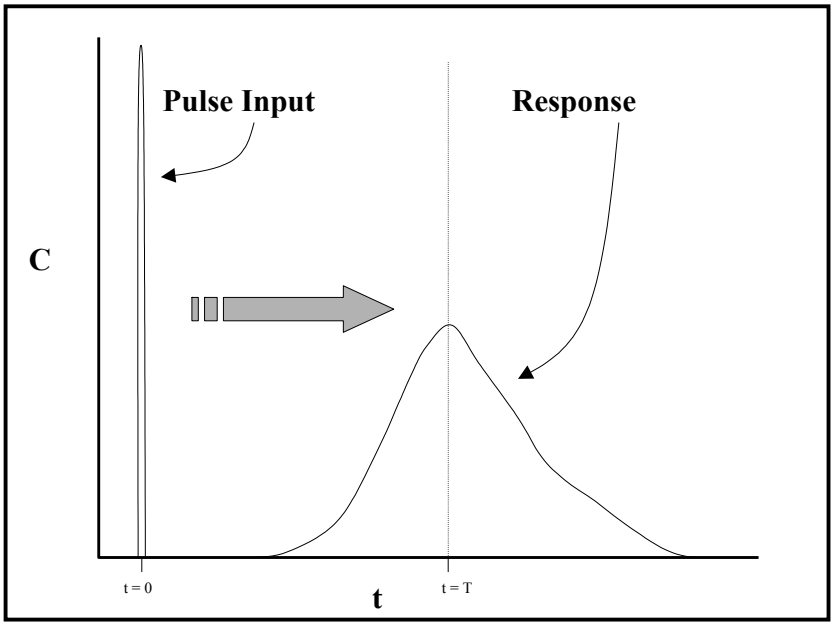

Figure E-3: Example of a response to a pulse input tracer test (Levenspiel, 1972).

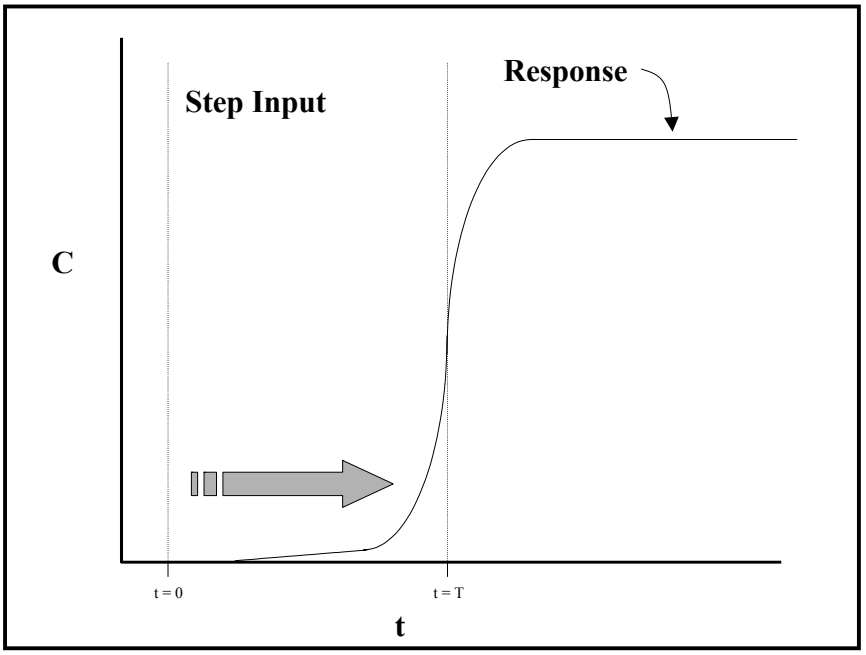

Figure E-4: Example of a response to a step input tracer test (Levenspiel, 1972).

E-2.4 Mathematical modelling of non-ideal hydraulic behaviour

In order to assess the non-ideal hydraulic behaviour of a reactor, the tracer response obtained form it is fitted to response curves fitted to various mathematical models and to compare these curves to curves predicted for ideal flow. It is also possible to calculate certain indices from the response curve

and to compare the values to the values known for ideal flow conditions. The two models most often used for simulating tracer response curves are the CSTRs-in-series model and the dispersion index model (Levenspiel, 1972).

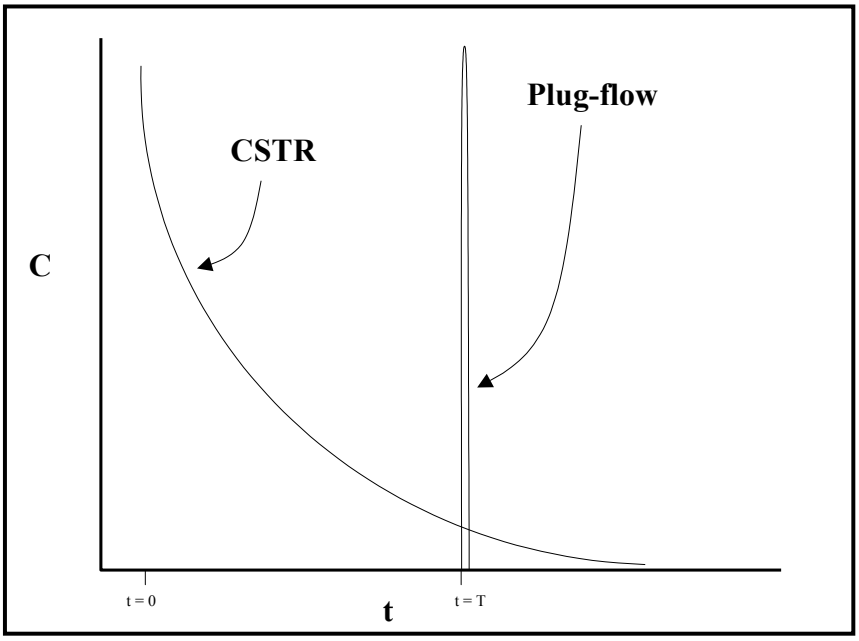

Figure E-5: Tracer response curves for a pulse input (Levenspiel, 1972).

E-2.4.1 The CSTRs-in-Series model

In the CSTRs-in-series model the actual reactor is simulated by a number (N) of ideal CSTRs in series (Levenspiel, 1972). The two extremes is characterised by N=1 (a CSTR) and N=∞ (a plug-flow reactor). The C-curve corresponding to a specific value of N can be determined using the following formulation:

$$C_\theta = \frac{N(N\theta)^{N-1}}{(N-1)!}e^{-N\theta}$$

Where:

C_θ	=	Normalised tracer concentration in the effluent (C/C_o)
C	=	Concentration
C_o	=	Effective concentration of injected tracer
θ	=	Normalised time (t/T)
t	=	time
T	=	Theoretical Residence time at design flow rate
N	=	The effective number of CSTRs (Levenspiel, 1972)

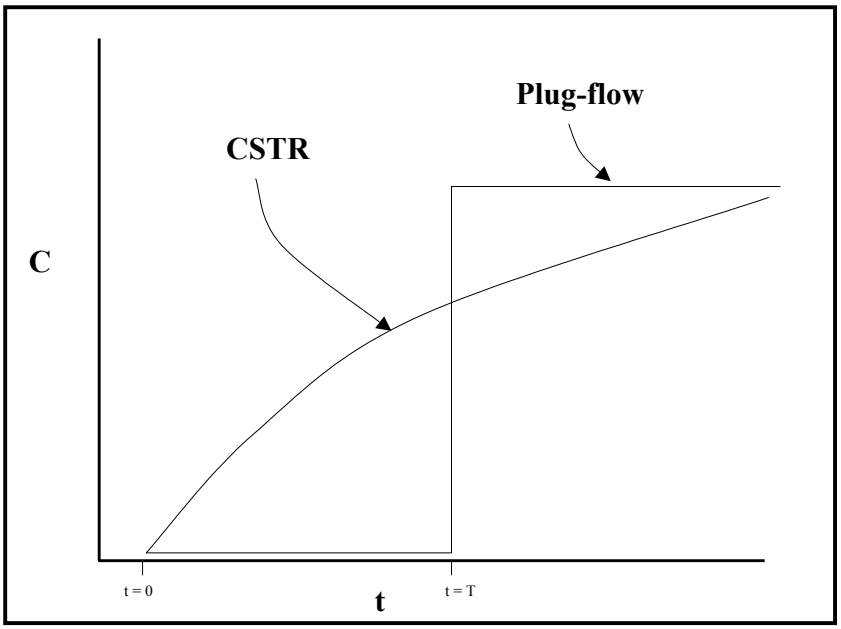

Figure E-6: Tracer response curves for a step input (Levenspiel, 1972).

C-curves for various values of N are shown in Figure 3.3. It is clear that the response approaches that of a plug-flow reactor as N increases in value. The relationship between a C-curve and a F-curve may be written as follows (Levenspiel, 1972):

$$F = \int_0^t C dt \quad \text{and} \quad C = \frac{dF}{dt}$$

This allows for easy transformation from one type of curve to the other. The choice of a step-input or pulse-input experiment is therefore based on the physical and chemical characteristics of the system under study (Levenspiel, 1972).

E-2.5 The dispersion index model

The dispersion index model provides an indication of the axial dispersion in an element of fluid as it moves through a reactor (Levenspiel, 1972). The axial dispersion in a plug-flow reactor is zero and in a CSTR it is infinite. The dispersion index is a dimensionless number that is defined as follows (Levenspiel, 1972):

$$d = \frac{D}{\mu L}$$

Where:
D = Axial mixing coefficient (m^2/h)
μ = Average flow velocity (m/h)
L = Length

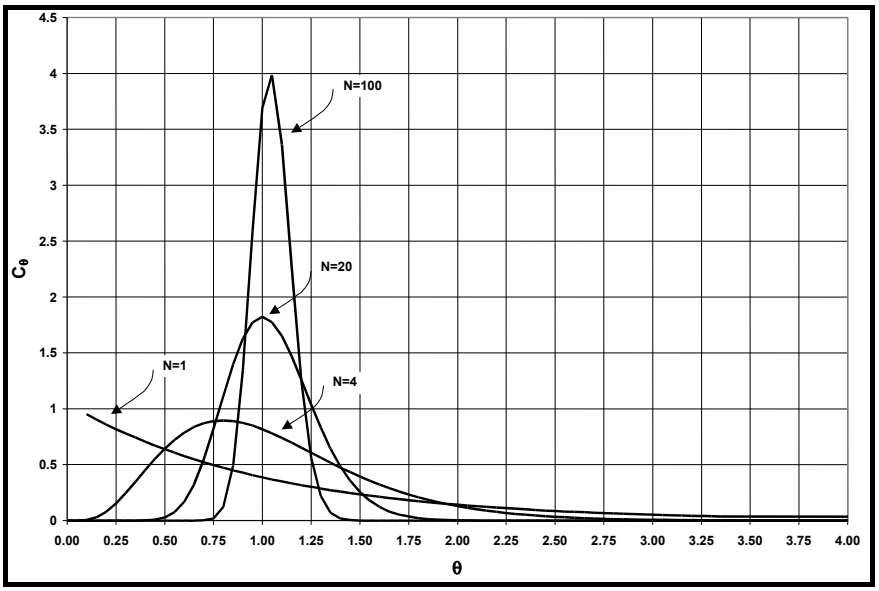

Figure E-7: C-curves for various values of N (Levenspiel, 1972).

The value of d can vary between 0 (perfect plug-flow conditions) and ∞ (a CSTR). The value of d can be obtained from a tracer response curve by way finding a solution of the following equation (Levenspiel, 1972):

$$\sigma_\theta^2 = 2d - 2d^2\left(1 - e^{\frac{-1}{d}}\right)$$

Where,

$$\sigma_t^2 = \left(\frac{\sum t^2}{\sum C}\right) - \left(\frac{\sum tC}{\sum C}\right)^2$$

and,

$$\sigma_\theta^2 = \frac{\sigma_t^2}{t^2}$$

This method is applied to the C-curve but could also be applied to a F-curve if it is transformed to a C-curve as a first step.

E-2.6 Injection of tracer

Tracer studies were performed on DPBR and SSRU1 (enhanced according to the IMPI Technology strategies) on the 7th of September 2005. A mass of 1.5 kg of sodium bromide (Analytical grade) was dissolved in 25 ℓ of AMD and poured into the front compartment of the splitter boxes of DPBR and SSRU2, respectively. The sodium bromide was therefore added as a pulsed input. Samples were taken of the effluent of the DPBR and SSRU2 starting one hour after tracer injection for a period of 17 d after tracer injection (Appendix F lists the sampling intervals and time of sampling after tracer injection). Samples were submitted to the PHD Analytical Facility for bromide determinations. Both

bioreactors are operated at a theoretical retention time of 68 h and flow was controlled using splitter boxes. Table E-1 compares the design and operation between SSRU2 and the DPBR.

Table E-1: The design and operation of SSRU2 and the DPBR.

Variable	SSRU2	DPBR
Configuration	Inverted truncated pyramid	Square
Waterproofing	Unit sealed with a manure layer followed by lining with plastic sheeting	Unit built with a double brick wall and plastered with cement containing waterproofing material and painted with two layers of Bitumen
Reactor size	23.3 m^3	25.9 m^3
Volume of carbon packed	18 m^3	18 m^3
Type of carbon packed	Layers of sawdust, sewage sludge, chicken litter, manure, hay	Layers of sewage sludge, pine wood chips, hay, chicken litter, sawdust, manure
Flow configuration	Down flow	Down flow
Molasses supplementation	Yes	Yes
Influent	Mixture of West Discharge and South Discharge water	Mixture of West Discharge and South Discharge water

E-2.7 Mathematical analysis

A mathematical analysis was conducted, using the “CSTRs-in-series model” to generate a predicted response curve. This curve was used as the basis of the experimental design and gave some indication of the amount of tracer to be used during the tracer study as well as the sampling programme.

APPENDIX F
SAMPLING SCHEDULE FOR TRACER STUDIES

The sampling programme followed during the tracer studies is summarised in Table F-1.

Table F-1: Sampling schedule and time of sampling after tracer injection

Date	Day	Time	Time after tracer was injected (h)	Date	Day	Time	Time after tracer was injected (h)
07-Sept 05	Wednesday	08h00	0	11-Sept 05	Sunday	08h00	96
		09h00	1			09h00	97
		10h00	2			10h00	98
		11h00	3			11h00	99
		12h00	4			12h00	100
		13h00	5			13h00	101
		14h00	6			14h00	102
		15h00	7			15h00	103
		16h00	8			16h00	104
		19h00	11			19h00	107
		21h00	13			21h00	109
08-Sept 05	Thursday	08h00	24	12-Sept 05	Monday	08h00	120
		09h00	25			09h00	121
		10h00	26			10h00	122
		11h00	27			11h00	123
		12h00	28			12h00	124
		13h00	29			13h00	125
		14h00	30			14h00	126
		15h00	31			15h00	127
		16h00	32			16h00	128
		19h00	35			19h00	131
		21h00	37			21h00	133
09-Sept 05	Friday	08h00	48	13-Sept 05	Tuesday	08h00	144
		09h00	49			09h00	145
		10h00	50			10h00	146
		11h00	51			11h00	147
		12h00	52			12h00	148
		13h00	53			13h00	149
		14h00	54			14h00	150
		15h00	55			15h00	151
		16h00	56			16h00	152
		19h00	59			19h00	155
		21h00	61			21h00	157
10-Sept 05	Saturday	08h00	72	14-Sept 05	Wednesday	08h00	168
		09h00	73			12h00	172
		10h00	74			16h00	176
		11h00	75	15-Sept 05	Thursday	08h00	192
		12h00	76			12h00	196
		13h00	77			16h00	200
		14h00	78	16-Sept 05	Friday	08h00	216
		15h00	79			12h00	220
		16h00	80			16h00	224
		19h00	83	17-Sept 05	Saturday	08h00	240
		21h00	85			15h00	244

Table F-.1: Sampling schedule and time of sampling after tracer injection (continued)

Date	Day	Time	Time after tracer was injected (h)
18-Sept 05	Sunday	08h00	261
		15h00	268
19-Sept 05	Monday	08h00	285
		15h00	292
20-Sept 05	Tuesday	08h00	309
		15h00	316
21-Sept 05	Wednesday	08h00	333
		15h00	340
22-Sept 05	Thursday	08h00	357
		15h00	364
23-Sept 05	Friday	08h00	381
		15h00	388

APPENDIX G
TERMS OF REFERENCE

G-1 AIMS

1. Verify the long-term performance and behaviour of the DPBR and enhanced SRUs at VCC for sulfate, metals and acidity removal.
2. Perform tracer studies on the DPBR.
3. Decommissioning and autopsying of the SRUs operated (9 Years) without any enhancement strategies in order to obtain a detailed chemical and microbiological (DNA extraction and molecular typing of microbial population) characterisation of the SRUs to establish and confirm the cause of system failure.

G-2 RESEARCH PRODUCTS

Report that:

- Verifies the long-term performance and behaviour of passive treatment plants for sulfate, metals and acidity removal
- Verify system malfunction through the decommissioning and autopsying of the SRUs operated without enhancement strategies.

G-3 COMMENCEMENT AND DURATION

From 01 April, 2005 to 31 March, 2006

G-4 PROJECT STAFF

Mr W Pulles	Project leader
Prof P Rose	Researcher
Dr SE Coetser	Researcher
Mr S Hlophe	Assistant
Michelle Bowker	Student

G-5 MOTIVATION

The South African mining industry is facing major problems with regard to the management and treatment of contaminated mine water. These problems exist with regard to operational mines and, importantly, they also exist for mines which have ceased operations and which have long term water quality problems.

Currently available effluent treatment technology for dealing with water quality problems is primarily of a chemical or physical nature. Although this technology is generally effective, it typically has very high capital and operating costs and intensive, ongoing, long-term maintenance requirements. This is a particular problem for those mines that have ceased operations and where it is not practical or cost-effective to construct an active treatment plant that requires constant supervision and maintenance.

An urgent need was, therefore, identified to develop low cost, self-sustaining, low maintenance passive treatment systems to address the problems of acidification and salinisation (in terms of sulfate) at operating, defunct and closed mines in South Africa. For this purpose, passive treatment is defined as follows:

A water treatment system that utilises naturally available energy sources such as topographical gradient, microbial metabolic energy, photosynthesis and chemical energy and requires regular but infrequent maintenance to operate successfully over its design life.

A very large research effort has been undertaken over the last 9 years in South Africa to develop passive water treatment technology that can reliably remove sulfates, acidity and metals from contaminated mine water. This has resulted in the development of the Degrading Packed Bed Reactor (DPBR) that has, in extensive laboratory tests, demonstrated the ability to perform at an efficiency rate that is some 800% higher than the best comparable passive treatment technology available anywhere else in the world. This led to the patented innovative Integrated Managed Passive Treatment (IMPI) Technology. The DPBR technology is a world leader by almost an order of magnitude and has evolved from a sustained 9-year research programme with a 2004 value of around R21 million.

A key feature of this technology is that it will generally be required to operate for a number of years, typically decades, and continue to perform in accordance with its specified duty. A key uncertainty that is repeatedly raised by the mining industry and the regulatory authorities relates to the confidence that can be placed in the long-term performance of the units. Research undertaken to date has demonstrated that the passive treatment plants do undergo a change in performance with time. Specifically, the plants operate at a high efficiency for the first 6-9 months before settling down to a lower but more sustainable efficiency over the long term. Monitoring data is, however, inconsistent and some reactors show increasing trends while other show decreasing efficiencies. Previous research has demonstrated the overall efficiency of the process is defined by the efficiency of the anaerobic hydrolysis of the solid lignocellulose material within the reactors. As part of a comprehensive Innovation Fund project, extensive fundamental research has been undertaken to understand the lignocellulose hydrolysis mechanism and led to the development of a patented DPBR and the IMPI Technology.

A WRC grant (Project 1348) facilitated the incorporation of the enhancement strategies of the DPBR to three of the existing sulfate removal units (SRUs) at the VCC Passive Treatment Plant while the rest of the SRUs continued to operate as normal. One SRU was decommissioned completely and rebuilt, repacked and operated as a DPBR while enhancement strategies were incorporated to another two SRUs. Results obtained to date have indicated that the DPBR upgrade is capable of unlocking lignocellulose material that is unavailable within the normal SRU systems. Increased performance in terms of sulfate reduction as high as 870% has been observed. These DPBRs have only been in operation for about 2 years and, given the need to demonstrate the ability to continue operating over long time periods, additional monitored operating time is critically required. A further WRC grant (Project K8/578) enabled the further continued evaluation of the enhancement strategies as well as the monitoring of the VCC Passive Treatment Plant for an additional year until March 2005.

The last remaining hurdle before implementing the technology in South Africa and internationally is to collect reliable operating data for plants operated under field conditions such as at the VCC pilot plant facility and to demonstrate the enhanced performance of the DPBR systems there. The VCC Passive Treatment Plant reactors which have been in operation for 8 years are believed to be the longest running, most closely monitored passive sulfate removal reactors. Without the enhancement strategies these SRUs have not been able to achieve the target sulfate loads removed required for passive treatment to be economically feasible and the have recently undergone a significant downward shift in their operating efficiency. However, these SRUs can not be summarily shut down without a thorough decommissioning and autopsy programme as it is critical to establish the cause of this shift. Detailed chemical and microbiological characterisation of the different carbon layers of the SRUs will enable the microbial identification of areas where active sulfate reduction occurred, the degradation rate of the different carbon layers as well as the hydraulics of the system.

This project will focus on the continued evaluation of the DPBR and enhancement strategies for another year, as well as a detailed chemical and microbiological characterisation of the SRUs operated without any enhancement strategies.

G-6 METHODOLOGY

Monitoring, evaluation and verification of long-term performance of the DPBR and SRUs operating with enhancement strategies

The DPBR and the SRUs operating with the enhancement strategies have demonstrated the ability to unlock carbon sources that are unavailable under normal operating conditions. This aspect, however needs to be confirmed through the ongoing monitoring of the modified reactors. Such long-term monitoring of these reactors is essential to give the confidence to design such reactors for long-term operation at abandoned mine sites and remote treatment locations.

The pilot plant operator from the local community will continue to be employed to monitor the plant and to collect the daily data. Monthly water samples will continue to be taken at key points on the plant or more detailed analysis. These samples will be analysed at PHD's dedicated passive treatment laboratory in order to reduce costs and to ensure more reliable data. Assessment programmes, in addition to the normal monthly sampling will be undertaken. The detailed operating and monitoring protocol that will be followed has already been developed and has been successfully applied at VCC during the preceding years.

The results of the research will be used to prepare a report that gives guidance on effective operating strategies that can be applied to DPBR passive treatment plants in order to enhance their long-term efficiency for sulfate, acidity and metals removal. This information is critical for the successful implementation of the passive treatment technology.

Tracer studies

Detailed tracer studies will be conducted on the DPBR and the 2 SRUs operating with the enhancements in order to determine the hydraulic performance of the systems.

Decommissioning, autopsying of SRUs operated without enhancement strategies in order to obtain a detailed chemical and microbiological characterisation for the cause of system failure

A detailed experimental programme will be developed to decommission these SRUs and undertake full autopsies of the SRU's contents and how they have changed relative to the starting conditions. Hydraulic studies performed on the SRUs indicated that only the cores of the SRUs were hydraulically active and therefore the study will focus on this section of the SRUs.

Liquid samples will be taken at different depths of the SRUs and analysed for sulfate concentrations, sulfide concentrations, pH, redox potential, COD concentrations, alkalinity, acidity, conductivity, nitrate concentration, fluoride concentration, chloride concentration and various metal concentrations etG.

Chemical characterisation of the solid carbon fractions sampled will be milled and subjected to cation exchange capacity determinations, dry matter content, ash content, protein content, fat content, fibre content, neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), *in vitro* digestibility, starch content, water soluble carbohydrates (WSC), total non structural carbohydrates (TNC), organic extraction using dichloromethane and subjected to GCMS, microscopy studies, XRD and XRF etG.

Aseptic samples will be taken, sealed and refrigerated for DNA extraction and molecular typing of the microbial populations.

The results obtained through microbiological and chemical analysis will be used to verify causes for system malfunction and also to establish the changes which occurred in the system since start-up.

G-7 WORK PROGRAMME

Monitor DPBRs and SRUs operating with enhancement strategies
Description: The daily monitoring for flow, temperature, conductivity and pH will continue by the on-site pilot plant operator. Data will continue to be submitted to PHD's offices on a daily basis for incorporation into the pilot plant database. Throughout this period, monthly samples will also be taken by PHD staff for analysis of sulfate, sulfide, CD, alkalinity, pH, conductivity, Fe, Al and Mn at PHD's laboratory. All collected data will continuously be added to the existing 8-year database for ongoing evaluation and assessment.
Deliverable: Brief report on performance and operation of passive treatment plant

Hydraulic performance of DPBR and SRUs operated with enhancement strategies
Description: The DPBR and the two SRUs operated with enhancement strategies will be injected with a tracer and effluent samples will be taken from the reactors over an extended period. Tracer concentrations will be determined and concentrations plotted over time. Results obtained will be used to describe the hydraulic performance of the reactors.
Deliverable: Brief report on the hydraulic performance of the DPBR and the SRU operated with enhancement strategies.

Decommissioning and autopsying of the SRUs operated without enhancement strategies
Description: A detailed chemical and microbiological characterisation of the SRUs not operated with enhancements strategies will be performed. Liquid and solid samples will be taken at different carbon layers. Liquid samples will be analysed for sulfate, sulfide COD, pH, redox potential, alkalinity and various metals. Solid carbon samples will be analysed in terms of various parameters to describe the

carbon profile present. Aseptic samples will be taken for DNA extraction and molecular typing of the molecular population.
Deliverable: Report on the chemical and microbiological characterisation of the SRUs not operated with the enhancement strategies. Changes occurring in the system from start-up and causes for system malfunction will be reported.

Monitor DPBRs and SRUs operating with enhancement strategies
Description: The daily monitoring for flow, temperature, conductivity and pH will continue by the on-site pilot plant operator. Data will continue to be submitted to PHD's offices on a daily basis for incorporation into the pilot plant database. Throughout this period, monthly samples will also be taken by PHD staff for analysis of sulfate, sulfide, CD, alkalinity, pH, conductivity, Fe, Al and Mn at PHD's laboratory. All collected data will continuously be added to the existing 8-year database for ongoing evaluation and assessment.
Deliverable: Brief report on performance and operation of passive treatment plant

Prepare final project report
Description: A report will be prepared to present the long term performance of the DPBR and the SRUs operated with the enhancement strategies.
Deliverable: Report on long term performance of VCC passive treatment in terms of the DPBR and the 2 SRUs operated with enhancement strategies.

APPENDIX H
CAPACITY BUILDING

The project employs a PDI pilot plant operator from the surrounding community in order to further his training as a passive treatment plant evaluator. Most of the chemical analyses are performed at the PHD Analytical Facility where all of the analysts are PDI's. The student employed at EBRU who participated in the project is also a PDI.

The capacity building objectives contained in the project proposal and contract were met and satisfied.