THE REFINING OF CALCIUM USING A SULFATE REDUCING BACTERIAL SYSTEM

THESIS

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by

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Abstract

White lime is used in many industries in South Africa but is not produced locally and must be imported. Many technologies have been suggested for the large-scale manufacture of calcium carbonate but these are not necessarily suitable for application in South Africa. This study investigated a chemical preparation of calcium carbonate combined with biological purification.

Calcium containing materials from the Pretoria Portland Cement, Lime Division factory at Lime Acres in the Northern Cape were studied as the starting materials for the manufacture. Investigation showed that they contained various impurities, including iron and manganese compounds which were largely responsible for the brown-grey colour of the lime products. Complete dissolution of calcium hydroxide, the purest of the potential starting materials, and subsequent hydroxide precipitation was not successful in removing all iron and manganese. Precipitation with sulfide ions was successful, decreasing levels of metals to below the detection limit of atomic absorption spectrophotometry.

Studies of all potential starting materials revealed that the levels of impurities in the starting material did not have a large effect on levels of impurities in the calcium carbonate produced. It was therefore possible to convert the residual calcium oxide or hydroxide in waste lime dusts to white calcium carbonate, a marketable product. Recycling of the water and starting material used in the process served to increase, rather than decrease, the purity of the calcium carbonate product. This allows for water conservation as water is not consumed in the process but merely utilised.

When waste lime dust was used as the starting material, sulfate was found in the product. While still a white lime, the calcium carbonate was not chemically pure. Sulfate removal was therefore investigated and the use of sulfate-reducing bacteria was studied as a novel application. A mixed sulfate-reducing bacterial population was isolated and found to be highly active at sulfate concentrations between 0.2 and 2 %. They were capable of

autotrophic growth and could reduce sulfate in solutions with elevated pH and in calcium carbonate suspensions, although they did not grow readily in these media.

A process was designed for the production of bulk quantities of calcium carbonate making use of the facilities and materials available at Lime Acres. This was tested using a small scale bench-top reactor series, with favourable results. The process would allow automatic, continuous production of large quantities of white lime using waste lime dust. Provision was also made for manufacture of smaller quantities of pure calcium carbonate using sulfate-reducing bacteria to remove the sulfate impurity.

Contents

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May you all reap one hundred-fold what you have sown

Ultimately, all thanks and glory go to God: words cannot express my gratitude.

Tables

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Abbreviations

PPC	Pretoria Portland Cement
$CaCO_3$	Calcium carbonate, commonly referred to as limestone
$Ca(OH)_2$	Calcium hydroxide, commonly referred to as slaked or hydrated lime
CaO	Calcium oxide, commonly referred to as unslaked lime
CO_2	Carbon dioxide
N_2	Nitrogen
$Ca(HCO_3)_2$	Calcium bicarbonate
NH_3	Ammonia
Ca^{2+}	Calcium ions
$CaSO_4$	Calcium sulfate
H_2S	Hydrogen sulfide
HCl	Hydrochloric acid
Mn	Manganese
Fe	Iron
HNO_3	Nitric acid
$NaHCO_3$	Sodium hydrogen carbonate
NaOH	Sodium hydroxide
Fe^{2+}	Iron(II) ions
Fe^{3+}	Iron(III) ions
Na_2SO_4	Sodium sulfate
H_2CO_3	Carbonic acid
FeS	Iron sulfide
SO_4^{2-}	Sulfate
S^{2-}	Sulfide
SRB	Sulfate reducing bacteria
CO	Carbon monoxide
H_2	Hydrogen
H_2O	Water

S	Sulfur
COD	Chemical oxygen demand
FGD	Flue gas desulfurisation
OH^-	Hydroxide ions
Mn_2O_3	Manganese oxide
MnO_2	Manganese dioxide
HS^-	Hydrogen sulfide
MnS	Manganese sulfide
Fe_2O_3	Iron oxide
K_{SP}	Solubility product
Ca^{2+}	Calcium ion
M^{2+}	Divalent metal ion
XRF	X-ray fluorescence spectrometer
LK6 c.d.	Lime kiln 6 cyclone dust
LK9 p.d.	Lime kiln 9 precipitator dust
SiO_2	Silica or silicon dioxide
Al_2O_3	Alumina or aluminium oxide
TiO_2	Titanium dioxide
P_2O_5	Phosphorus pentoxide
SO_3	Sulfur trioxide
Cl	Chloride
K_2O	Potassium oxide
Na_2O	Sodium oxide
Ag	Silver
AgCl	Silver chloride

Chapter 1: The Production of White Lime

1.1 The Lime Industry

1.1.1 The Lime Industry in South Africa

"Limestone and its derivative, lime, probably find more applications in industry than any other natural product" (Martini and Coetzee, 1976)

Limestone is a calcium carbonate rock containing varying amounts of magnesia, ferric oxide, alumina and silica. It is used in the manufacture of cement, as a flux in smelting operations, as a neutraliser in acid soils and as a building stone (Martini and Coetzee, 1976). When this limestone is calcined, heated in order to drive off the carbon dioxide, calcium oxide is formed. This is commonly referred to as lime. Lime is used in the manufacture of gold, uranium, steel, paper, sugar, platinum and ferro alloys. It is also widely used for water purification and has some applications in the building industry including serving to modify clay soils, giving them the strength to carry roads, parking lots or buildings (PPC Lime, 1987).

There are many limestone deposits in South Africa and most of those presently mined supply cement factories in their general vicinity. Limestone quarries at Dwaalboom, Beestekraal, De Hoek, Ongegund, Loerie, Slurry, Lichtenburg and Dudfield (Martini and Coetzee, 1976) supply the Pretoria Portland Cement (PPC) factories of Dwaalboom, Jupiter, Hercules, De Hoek, Riebeeck West, Port Elizabeth and Slurry and the Alpha and La Farge (previously Blue Circle) cement factories. High-grade calcitic limestone and lime are produced by Idwala Lime (previously Alpha) and PPC Lime whose factories are in the Northern Cape at Danielskuil and Lime Acres respectively (Creamer, 1998). PPC Lime and ISCOR produce dolomitic lime. The main limestone quarries and lime factories mentioned above are shown in figure 1.1.

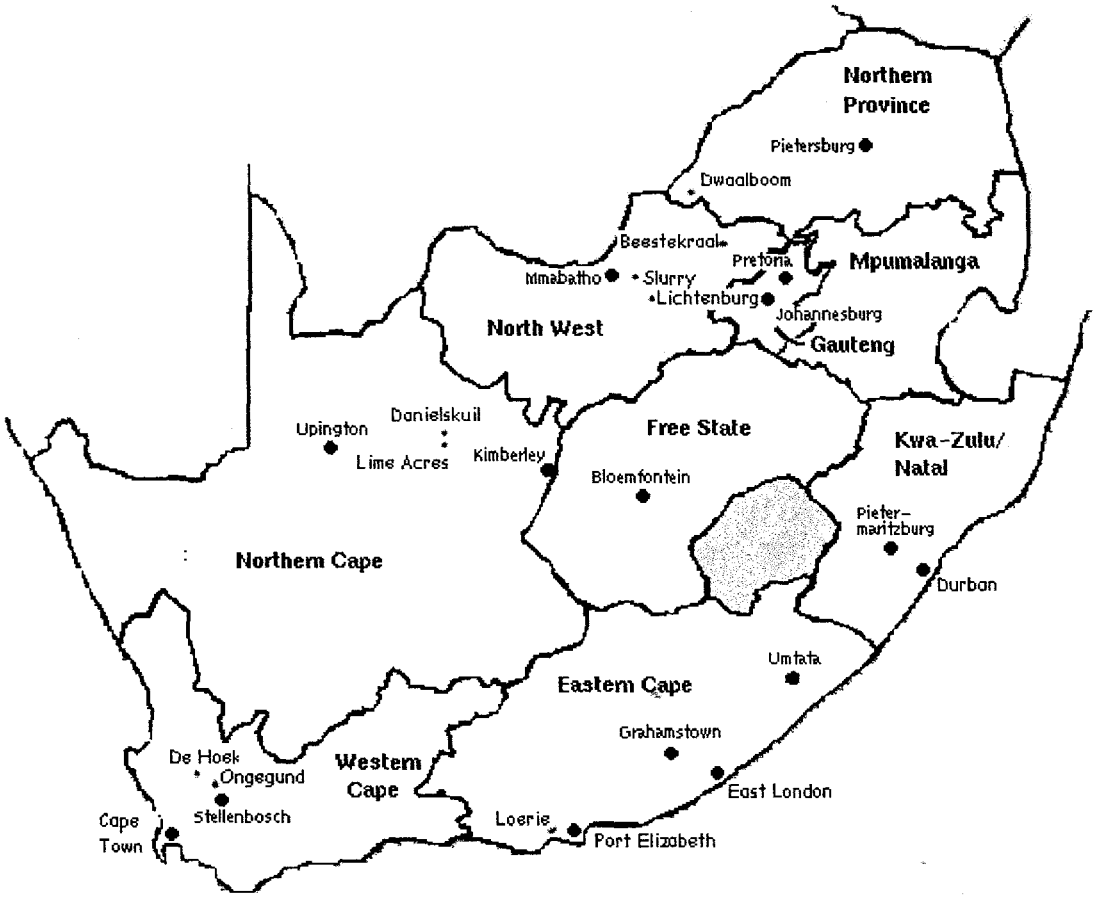

Figure 1.1 Location of limestone quarries and lime factories in South Africa

1.1.2 The Manufacture of Lime

The process of lime manufacture from limestone is shown in the flowsheet (Figure 1.2). Briefly, limestone is mined from a quarry and is transported to crushers. Primary and secondary crushers are followed by screening operations, ensuring that only limestone of the correct size enters the kiln. Lime is produced in either a vertical or rotary kiln by calcination of the limestone. Combustion of fuel provides the high temperatures necessary to complete this process. The lime emerging from the calcining zone of the kiln is cooled and may then be sold as lump lime. Alternatively, the lime is ground to produce ground unslaked lime or is hydrated in a controlled manner and sold as slaked lime (calcium hydroxide) (Boynton, 1980).

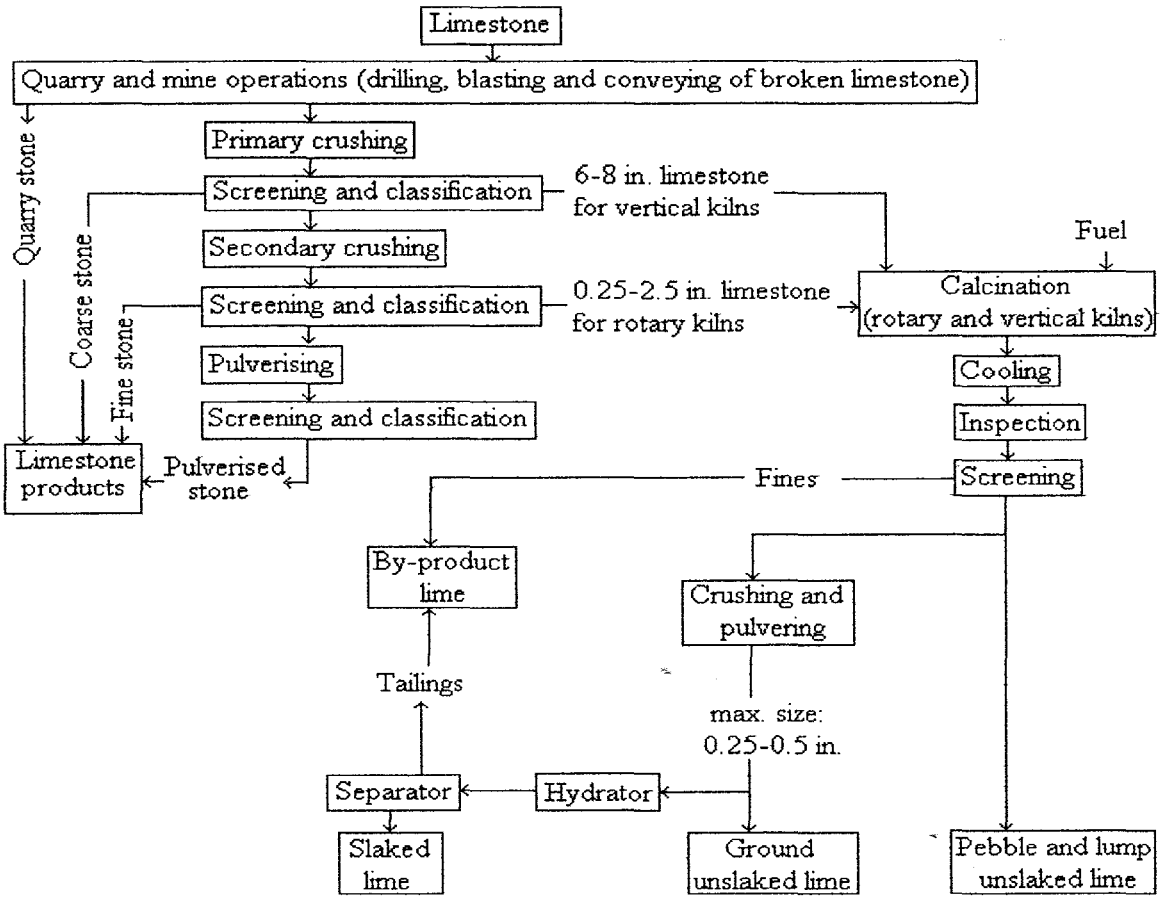

Figure 1.2 Flowsheet of an integrated lime operation

1.1.3 White lime

Reagent Grade $CaCO_3$ and $Ca(OH)_2$ are white powders but natural limestone and lime are usually grey to brown in colour. Minor constituents (especially iron and manganese containing compounds) of the natural limestone are the major contributors of this colour. For most industries, the value of the lime or limestone is simply its ability to neutralise acid and the presence of small levels of highly coloured impurities is unimportant. Increasingly, however, the global lime market is moving toward high quality and high value products and white lime is one of these (Gori, 1998). The term "white lime" is used non-specifically in this sense to include white $CaCO_3$, $Ca(OH)_2$ and CaO.

White calcium carbonate is used in many applications. It is used as a filler in the manufacture of paper, rubber, electric insulators and plastics and also has a role in the paint industry where it is used as a pigment. In these applications, it is widely replacing china clay and titanium dioxide because of the properties it can give to the end user's product. A paper mill, for example, may be willing to pay a premium price for a filler which allows it to use a lower cost pulp while maintaining the paper quality (Gori, 1998). Calcium carbonate also has a market in pharmaceutical applications where it is used mainly in antacid tablets and toothpaste. The purity of calcium carbonate intended for human consumption is obviously very important.

1.1.4 Lime Acres

Pretoria Portland Cement (PPC), Lime Division has a lime factory situated at Lime Acres in the Northern Cape 160 km west of Kimberley. It occurs within the Lime Acres Member of the Ghaapplato Formation, Cambell Group, Griqualand West Sequence. Just over 1 million tons of quarry material is mined every month and the high grade portion is crushed and screened. Some of the crushed limestone is sold to Rand Water Board and a few sugar mills where limestone is burned (PPC Lime, 1987). The rest of the usable fraction is calcined in nine rotary lime kilns where the calcium carbonate decomposes to calcium oxide and carbon dioxide. Other components of the limestone are oxidised in the kiln to their highest stable oxidation state.

The benefit of having multiple kilns is that different kilns can be used to produce different forms of lime. The steel industry, for example, requires a "soft burnt" porous lime whereas the carbide industry uses a "hard burnt" dense lime. Kiln conditions can be manipulated to produce these variations. Some lime is sold as ground unslaked lime and some is hydrated and sold as slaked lime (PPC Lime, 1987).

Not all material entering a kiln leaves the kiln as lime. During the passage of the limestone through a kiln, dust is generated. This dust is drawn with the hot air, to the back end of

the kiln. Discharge of this dusty air would harm the environment and it is therefore passed through cyclones and precipitators. A cyclone dust collector is a conical vessel and air and material that enter follow a spiral path toward the bottom. Medium to large dust particles settle out in the cyclone and are removed while the air carrying the fine dust particles is directed into an electrostatic precipitator. Here, a discharge electrode imparts a negative charge to the dust, causing it to be attracted to large collecting plates. The dust clings to the plates until a hammer mechanism hits the plate and the dust falls into collection vessels below (Boynton, 1980 and Gaylard, 1994). Both the cyclone and precipitator dusts consist of some calcined limestone as well as lighter impurities. This dust is discharged onto waste heaps and is at best useless and at worst an environmental hazard.

Of the commercially useful products manufactured at Lime Acres, none is chemically pure. Table 1.1 shows a typical chemical analysis of the limestone mined from the quarry at PPC Lime Acres as well as the lime products made using this starting material (PPC Lime, 1994).

Table 1.1 Typical chemical analysis of limestone and lime

% Element oxide	Limestone ($CaCO_3$)	Lump lime (CaO)	Ground unslaked lime (CaO)	Slaked lime $Ca(OH)_2$
Ca (as CaO)	53.9	94.0	92.0	72.0
Mg (as MgO)	1.3	2.0	2.0	1.2
Silica (as SiO_2)	<0.01	1.5	2.0	1.0
Mn (as Mn_2O_3)	0.7	1.0	1.2	0.5
Fe (as Fe_2O_3)	0.2	0.5	0.5	0.3
Al (as Al_2O_3)	0.1	0.3	0.3	0.8
CO_2	43.6	1.0	2.0	1.0
Free carbon as C	0.04	<0.01	<0.01	
Sulfur as SO_3	0.2	<0.03	<0.03	
P (as P_2O_5)	<0.01	<0.01	<0.01	

It can be seen that there are impurities in the limestone mined and this translates to impurities in the lime produced. The natural limestone is not of a sufficiently high purity for sale as white lime and it is necessary to import white lime to supply the local market which has been estimated at 100 000 tonnes per annum. Local manufacture of this product would be economically beneficial.

1.2 Production of White Calcium Carbonate

Many different patents for the production of white calcium carbonate ($CaCO_3$) have been filed covering either the production of synthetic calcium carbonate or the purification of natural limestone. These can be grouped according to the starting materials used and the methods employed.

1.2.1 Natural Limestone

Natural limestone deposits have different levels of impurities. Some natural deposits are sufficiently free of coloured impurities to be mined and used with no further modification. Other deposits are beneficiated. It has been found that when a fairly pure limestone (97.1 % $CaCO_3$) is calcined at 950°C for 2 hours, slaked and carbonated using 12% CO_2 in N_2, the resulting $CaCO_3$ is 98 % pure (Madigan, 1956). It has also been found that $CaCO_3$ particles found in lime sludge are larger and have a greater specific gravity than contaminating particles and can be separated using centrifugation (Kyosai and Murakami, 1976). Separation using flotation processes has been suggested (for example Shiftlet, 1987). Coarse crushed limestone has been added to a molten mixture of Na_2CO_3 and K_2CO_3 at 750°C in a 1:1:1 ratio. The $CaCO_3$ dissolved whereas the impurities were insoluble. The melt was poured into water, allowing the $CaCO_3$ to be collected by filtration and the filtrate was evaporated to dryness and the salts returned to the melt (Cunningham and Finn, 1956). Reductive bleaching agents are sometimes used in processes starting with natural limestone (Horikoshi *et al.*, 1994). Most methods for the production of white $CaCO_3$ however, involve chemical reactions.

1.2.2 Carbonation of a $Ca(OH)_2$ Solution or Suspension

The first group of methods involve the dissolution or suspension of $Ca(OH)_2$ in water and the carbonation of this solution. Wood filed a patent for this process in 1927 (Wood, 1927). This basic process has often been altered to prepare a product suited to the application (Shimosato and Aiura, 1952; Schur and Levy, 1953; Kimmel and Tilicã, 1958; Farbenfabriken Bayer A. -G., 1965; Toa Gosei Chemical Industry Co., Ltd., 1967; Ilysov *et al.*, 1968; Arav *et al.*, 1971; Postoronko *et al.*, 1971; Novotny, 1972; Miki *et al.*, 1972; Hoshi *et al.*, 1973, Tahara *et al.*, 1973a; 1973b; Harris, 1975; Shibazaki *et al.*, 1978; Murakami *et al.*, 1978; Imperial Chemical Industries Ltd, 1978; Bulat *et al.*, 1980; Gospodaru *et al.*, 1981a; Park *et al.*, 1989; Valiullin *et al.*, 1990; Bleakley and Jones, 1992; Zhang *et al.*, 1994; Domka, 1996; Bunger *et al.*, 1997; Unitecknik-Thurner Anlagenbaugesellschoft, 1997).

Studies of the kinetics of the carbonation have been performed (Gochev and Mishonova, 1969). Barrett added small amounts of alkali carbonates, hydroxides or bicarbonates and so manipulated the particle size distribution (Barrett, 1949). Small additions of formalin, sodium carbonate or bicarbonate have also been shown to accelerate the reaction (Ohno, 1952). Addition of N nitrilotriacetate gave a finely divided product suitable for a rubber filler (Farbenfabriken Bayer A. -G., 1962) as did small additions of water soluble diamines (Taylor, 1967) and phosphate alkali salts (Suzukawa *et al.*, 1974; Valiullin *et al.*, 1988). Addition of organic acids has also been shown to affect the hardness and texture of particles formed (Woode, 1975). A number of patents have looked at the effect of minor additions (Novikov *et al.*, 1971; Vanderheiden, 1982; Filipescu and Enculescu, 1985; Zikmund *et al.*, 1987a; Frank *et al.*, 1998) and adding CO_2 as a liquid instead of a gas has been shown to produce a product with increased brightness and opacity (Wallace *et al.*, 1991). A review of the effect of process parameters and some additives has been written (Ovechkin *et al.*, 1963).

The presence of small particles of calcined limestone in the exit gas of a lime kiln has potential in $CaCO_3$ production. In one application, the exit gas was mixed with a fine spray of water to give slaked lime which was then carbonated using the CO_2 present in the scrubbed gas (McAllister, 1952). Process parameters and reactor design for similar processes have also been suggested (Gavrilescu, 1964; Laine *et al.*, 1981; Gospodaru *et al.*, 1981b; Todaka *et al.*, 1988a; 1988b; Nakajima and Saito, 1993; Li, 1994; Huege, 1997). In more recent applications, the aim of this process is less to produce $CaCO_3$ and more to remove CO_2 from the gas emitted from the kilns as legislation is now in place to limit CO_2 emission to the environment (Shibazaki *et al.*, 1995).

$CaCO_3$ is formed by the reaction:

$$Ca(OH)_2 + CO_2 \rightarrow CaCO_3$$

$CaCO_3$ is only slightly soluble in water so the product is easy to capture by filtration. In the presence of excess CO_2 however, the reaction continues:

$$CaCO_3 + H_2O + CO_2 \rightarrow 2\ Ca(HCO_3)_2$$

Calcium bicarbonate is soluble. This means that monitoring the extent of the reaction is important in maximising the yield and different ways of doing this have been devised.

In one reactor, CO_2 is introduced into a $Ca(OH)_2$ suspension and $CaCO_3$ is formed on the surface of $Ca(OH)_2$ particles. This results in a drop in pH and at a certain level, the CO_2 infection is halted. Continuous recycle of part of the suspension results in turbulence which causes the $CaCO_3$ to be removed. The $Ca(OH)_2$ surfaces are exposed again, the pH rises and CO_2 is re-introduced into the system (Kosin and Andrews, 1989). Electrical conductivity monitoring has also been used (Takuma, 1991).

1.2.3 Overcoming the Low Solubility of $Ca(OH)_2$ in Water

One of the limitations of the above processes is the low solubility of $Ca(OH)_2$ in water. If one uses a solution, the yields will be relatively small and if a suspension is used, one risks incomplete conversion to the carbonate. One way of alleviating this problem has been the use made of the fact that the solubility of $Ca(OH)_2$ in water is greatly increased in the presence of certain chemicals.

$Ca(OH)_2$ is soluble in solutions of ammonium salts (Weast and Astle, 1981). In 1944, Foster filed a patent for the production of $CaCO_3$ by bubbling a gas containing CO_2 into a lime slurry containing ammonia. Once all of the lime was converted, the solution was separated from the precipitated $CaCO_3$, which was dried (Foster, 1944). The ammonia or ammonium salts can be re-used (Murakami *et al.*, 1973; Tanaka, 1973; Takayama, 1977; Sivokhin, 1988). The precipitated $CaCO_3$ resulting from this type of process has been measured as having purities of 99.99 % (Sonoda *et al.*, 1987). Ammonia emissions from these processes can be decreased by absorbing the NH_3 in an NH_4HCO_3 solution. The $(NH_4)_2CO_3$ is a valuable by-product (Ryabukha *et al.*, 1989).

The improved solubility of $Ca(OH)_2$ in glycerol solutions is also of benefit (Hasegawa *et al.*, 1973) as is that with polyhydric alcohol additions (Tanaka *et al.*, 1978).

Another way to overcome the low solubility of $Ca(OH)_2$ in water is to perform the reaction in the solid state. Extremely fine $CaCO_3$, suitable for use in the rubber and paint industries, was produced by carbonating CaO in the presence of water vapour at elevated temperature (Souren, 1957). Yet another process used a bed of CaO fluidised with superheated steam to form a $Ca(OH)_2$ coating which was stripped from the grains and carbonated (Taizo and Kamatsu, 1973). Other processes treated $Ca(OH)_2$, calcined $Ca(OH)_2$ or calcined $CaCO_3$ with CO_2 at 300 - 800°C with a lower cost and shorter preparation time than conventional wet procedures (Suzuki, 1993).

1.2.4 Production of Calcium Carbonate from Natural Source Materials

Dolomite is a calcium, magnesium carbonate and many processes have been developed for the extraction of $CaCO_3$ and magnesium compounds from this starting material (Licencia Talamanyokat Ertekesito Vallalat, 1965; 1967; Suciu *et al.*, 1975; Liaz-Ur-Rahman *et al.*, 1981; Imai, 1988). Purities of 99.8 % have been reported for $CaCO_3$ produced in this way (Chryzanowski *et al.*, 1983). One way of separating the calcium from the magnesium component is by calcining the dolomite and then reacting the ground material with a sucrose solution to form soluble calcium sucrate. The suspension is filtered, removing the magnesium compounds and other insoluble material, and the filtrate is carbonated with CO_2 recovered from the first calcining step and the sucrose solution is recycled (Judd, 1967). Extraction with ammonium salts has also been reported (Kaminskas *et al.*, 1987; Zou, 1991) as has extraction into an organic phase (monoethanolamine) with subsequent carbonation (Zikmund *et al.*, 1987b; 1989). The separation has also been achieved by adsorbing the Ca^{2+} ions onto an ion-exchange resin, washing the resin with water to remove traces of Mg^{2+}, resuspending the resin and treating it with CO_2 in water (Iso and Saeki, 1996).

Gypsum ($CaSO_4.2H_2O$) and synthetic $CaSO_4$ are also popular starting materials. In 1935, Perel'man and Rozler published a method in which gypsum was treated with $(NH_4)_2CO_3$ and the precipitated $CaCO_3$ was collected by filtration. The use of phosphogypsum (a by-product in the production of phosphoric acid) resulted in a poorer quality product (Perel'man and Rozler, 1935). A process has also been designed in which the reactants are passed through a series of reaction vessels and a $CaSO_4$ conversion of 99 % has been achieved. Purification by froth flotation has been used (Robinson, 1953a; 1953b). Gypsum can be mixed with $NaHCO_3$ and carbonated (Mourad, 1955) or with concentrated Na_2CO_3 (Debara, 1975). $(NH_4)_2CO_3$ has also been used for the carbonation with the concomitant production of ammonium sulfate (Andrianov *et al.*, 1978; Gottschalk *et al.*, 1985; Hofmann *et al.*, 1989; Smelik *et al.*, 1992). CO_2 and NH_3 gas can also be substituted for the ammonium carbonate (Gorecki *et al.*, 1982, Mohanty *et al.*, 1993,

1994). Phosphogypsum can be treated with ammonium hydroxide and CO_2 with the same end products (Schroeder *et al.*, 1981).

It is possible to produce both sulfur and $CaCO_3$ from gypsum. In this case, the gypsum is calcined and slurried, the liquid and solid phases are separated by centrifugation and the liquid is carbonated. The H_2S liberated is converted to sulfur by the Claus-Chance process (Batista de Queiroz and Peres, 1984; Legey and Rodrigues de Moraes, 1986). Thermal decomposition of a $CaSO_4$ waste at 600 - 900°C in the presence of CO_2 results in the production of $CaCO_3$ (Kostyl'kov *et al.*, 1981).

$CaCO_3$ is a by-product in the beneficiation of phosphate ores. In this case, the phosphate ore is ground, roasted and treated with an aqueous solution of NH_4Cl. The suspension is filtered and the $CaCl_2$ found in the filtrate is treated with CO_2 and NH_3 to give a $CaCO_3$ precipitate and an NH_4Cl solution for recycle (Soderec, 1971; Mori, 1975; Lu, 1986). In the production of phosphoric acid, phosphate ore is treated with HCl and the $CaCl_2$ containing filtrate is first treated with $Ca(OH)_2$ to remove impurities (aluminium, iron, fluoride and silica) and then carbonated (Israel Mining Industries, 1972). NH_3 has also been used in place of $Ca(OH)_2$ to raise the pH above 9 to precipitate Mg, Mn and Fe. NH_3 and CO_2 are used to form NH_4CO_3 which is mixed with the $CaCl_2$ solution to precipitate $CaCO_3$. The remaining solutions are recycled (Lin, 1995)

In the manufacture of fertilisers, HNO_3 is used, resulting in a $Ca(NO)_3$ solution. This is treated with CaO, NH_4OH, $Mg(OH)_2$ or $Ca(OH)_2$, filtered and carbonated (Glaser and Vidensky, 1987a) or treated with $(NH_4)_2CO_3$ to give a pure product (Dmitrievskii *et al.*, 1990). The $Ca(NO_3)_2$ solution can be treated with CO_2 and NH_3 from waste urea hydrolysis to yield NH_4NO_3 and $CaCO_3$ (Brunborg, 1982). When certain purification steps are incorporated into the process, the $CaCO_3$ is sufficiently pure for use in the cosmetic, pharmaceutical and food industries (Havranek *et al.*, 1991).

1.2.5 Production of Calcium Carbonate from Chemicals

Bertrand and Leroy produced an extra light precipitated $CaCO_3$ through the reaction of $Ca(SH)_2$ and Na_2CO_3. The NaSH remaining was carbonated and the Na_2CO_3 recovered with the release of H_2S. This gas was bubbled through a slaked lime solution to form $Ca(SH)_2$ which was recycled (Bertrand and Leroy, 1951). A similar process used CO_2 to carbonate the solution (Collin, 1980). $CaCO_3$ can be recovered from CaS wastes originating from coal gasification. The CaS is reacted with H_2S, complexed with an alkanolamine, to give $Ca(SH)_2$ which is carbonated to give $CaCO_3$ and the complexed H_2S solution is recycled (Brooks and Lynn, 1997).

$CaCO_3$ with a high porosity and specific area was produced through the reaction of $CaCl_2$ and Na_2CO_3 with a glassy sodium polyphosphate addition (Clark and Thurston, 1952).

When $CaCl_2$ is passed through a strongly acidic ion-exchange column, the calcium is retained and HCl is eluted. Treating the resin with ammonium acetate elutes calcium acetate which can be carbonated to produce $CaCO_3$. The resin is regenerated with a dissociated H-containing acid (Barthel and Felix, 1997).

Aqueous $Ca(OH)_2$ and Na_2CO_3 have been mixed to produce a slurry of $CaCO_3$ and NaOH. The fine $CaCO_3$ was separated and used in paper filling and coating (Adler and Denholm, 1993).

NH_4CO_3 containing NaCl and a $CaCl_2$ solution were stirred vigorously to produce a bulky, very white $CaCO_3$ for use in the paper and plastics industry (Zoellner *et al.*, 1972). When the sodium salt is not included, phosphor-grade $CaCO_3$ may be produced (Heytmeijer *et al.*, 1978; Glaser and Vidensky, 1987b) and purification by ion current electrodialysis to remove the excess chloride is also possible (Tian *et al.*, 1984). The concentration of free alkali in the product has also been decreased by pre-treating the water, passing it through a magnetic field of induction (Valiullin *et al.*, 1988).

Spheroidal-shape reticulated microparticles, which have improved pigmentatory properties, are produced by the mixing of two water-in-oil emulsions containing $CaCl_2$ and K_2CO_3 (Erneta, 1974).

$CaCO_3$ with a high level of purity is often prepared by the carbonation of $Ca(NO_3)_2$, frequently in the presence of NH_3 (Chemiche Fabrik Kalk G.m.b.H., 1965; Eto, 1989; Fouche, 1993; Fouche, 1996; Ramsey, 1996). These procedures are typically complicated with many purification steps. An example is given below:

A highly pure $CaCO_3$ was prepared from a $Ca(NO_3)_2$ solution which was treated with $(NH_4)_2S$ for two days, boiled to remove the excess precipitating agent and filtered to remove the precipitate. The filtrate was then acidified with HNO_3 and further treated to remove iron and heavy metals traces. Bromine water was used to oxidise the Fe^{2+} to Fe^{3+} and excess oxidising agent was removed by boiling. The solution was cooled and 25 % NH_3 was added to bring the pH to 7.6 and saturated $(NH_4)_2CO_3$ was introduced to precipitate 5 % of the calcium. The suspension was heated for 20 - 30 minutes at 80°C, left to settle for 2 - 3 hours and filtered through ashless, iron-free filter paper. The main fraction of the $CaCO_3$ was precipitated from the filtrate with saturated $(NH_4)_2CO_3$, was washed with water (containing less than 10^{-8} % iron) to a negative reaction for NO_3^- and dried at 100 - 110°C. The product contained 4 x 10^{-6} % iron and less than 1 x 10^{-5} % heavy metals and manganese (Angelov and Khainson, 1956).

Other procedures involving a number of purification steps have been proposed (Papageorgios and Lobacz, 1973; Gol'dinov *et al.*, 1976; Langelin, 1976; Smolik and Ogiolda, 1979, Karakhanyan *et al.*, 1983; Ryabukha *et al.*, 1985). Double recrystallisation of the product also improves the quality (Ryabukha *et al.*, 1986). Technical grade $Ca(NO_3)_2$ has even been precipitated as calcium oxalate and the thermal decomposition (490°C - 540°C) of the washed precipitate gave reagent grade $CaCO_3$ (Ryabukha *et al.*, 1987). Lime has also been reacted with acetic acid to give calcium acetate which was then

converted into calcium oxalate and calcined to $CaCO_3$ at 750 - 800°C (Miltzlaff *et al.*, 1987).

$CaCO_3$ was purified by adding a 3 % aqueous solution of Et_2NCS_2Na to a neutral solution and adsorbing the metal complexes formed and the excess reagent on active carbon (Angelov, 1956). Another purification method involved passing the calcium salt solution through an ion-exchange resin, resulting in a decrease in levels of metals as well as SO_4^{2-} and S^{2-} through replacement of the NO_3^- in the column (Nakhodova *et al.*, 1966). $CaCO_3$ suspensions have also been purified with resin acid (Kanegafuchi Chemical Industry Co., Ltd. Jpn., 1980). Technical grade calcined lime when leached with an aqueous 20 % HCO_3NH_4, filtered hot and the filtrate treated with CO_2 and NH_3, resulted in a product greater than 99 % pure (Zikmund, 1982).

1.2.6 Manufacture of Calcium Carbonate Using Waste Products as Starting Materials

There is much interest in converting wastes into products of value. Internationally, disposal of waste is becoming increasingly expensive as strict regulations are imposed. These regulations restrict the types of wastes that may be dumped and there are also sometimes financial considerations. In addition, many companies are adopting the ISO 14 000 environmental management philosophies and retention of this certification requires constant improvement in terms of waste treatment. Many industries have developed complex waste treatment methods that allow them to produce commercially useful chemicals from their waste streams.

$Ca(OH)_2$ has been recovered through suspending a carbide sludge in water. This can be carbonated to produce precipitated $CaCO_3$ (Nakamura, 1952; Basargekar and Rare, 1980; Li and Tang, 1992; Casado, 1995; Sontag, 1996). A 20 % sucrose solution has also been used to improve the efficiency of this process and the sucrose is recycled (Nishikawa, 1975). Similarly, the addition of stoichiometric amounts of an ammonium salt to improve

the extraction efficiency, has been reported (Suryanarayana *et al.*, 1986; Cremer and Holz, 1986; Yuan, 1992). The CaC_2 has also been decomposed using mono- or diethanolamine and an acid (HCl, HNO_3, formic or acetic acid) with good results (Zikmund and Macho, 1992).

The waste from caustic soda plants has been treated with CO_2 to form $CaCO_3$ (Selfo and Gaxhenji, 1971) as has the water used to cool molten converter slag (Kaneko, 1989).

Waste distillery liquid from the soda industry has been treated with a Na_2CO_3 solution in the presence of $NaHCO_3$ and various additives (Postoronko, 1976a; 1976b; 1976c; 1976d; 1977; 1979; Postoronko and Rivinyi, 1979; Rivinyi and Postoronko, 1979; Postoronko, 1980). In another process, the waste liquor was converted to MgO and $CaCl_2$. An intermediate in this process, $MgCO_3$, is treated with an aqueous solution of $CaCl_2$ for the production of $CaCO_3$ and $MgCl_2.6H_2O$ (Suciu and Man, 1982). In the process developed by Craiu and collegues, the final waste liquor from the ammonia recovery distillation column and the Na_2CO_3 and NH_4Cl bearing residual solutions from the filtration separation of $NaHCO_3$, were mixed in the ratio 0.05:1 to 0.2:1 to precipitate $CaCO_3$. The supernatant liquid (containing NH_4Cl and $CaCl_2$) was treated with aqueous slaked lime and recycled to the distillation column for recovery of ammonia. Relative to the conventional ammonia-soda process, this design permits reduced consumption of lime, enhanced recovery of NH_3 and recovery of $CaCO_3$ without any additional consumption of energy or materials. This had not previously been attempted (Craiu *et al.*, 1982). Process parameters have been investigated (Trypuc and Buczkowski, 1990). $CaCO_3$ has also been precipitated from the $CaCl_2$ solution with $(NH_4)HCO_3$, $(NH_4)_2CO_3$ or NH_3 and CO_2 (Glaser and Vidensky, 1987c) with recycling of the CO_2 and remaining solutions (Glaser *et al.*, 1987). It has been reported that $CaCO_3$ produced in this way is pure enough for pharmaceutical applications (Nastachowski *et al.*, 1991; Trypuc *et al.*, 1992a; Trypuc *et al.*, 1992c) although the iron and sulfate must be removed during the process (Trypuc *et al.*, 1992b).

Waste sludges of alumina production were treated with dilute HCl to leach the calcium ions. Carbonation with ion-exchange membranes was subsequently used to produce $CaCO_3$ and regenerate the HCl (Gol'dman *et al.*, 1978). The mixture of waste calcium solutions with waste $Mg(HCO_3)_2$ solutions under suitable conditions also resulted in $CaCO_3$ (Raschman, 1988). Ammonium bicarbonate and ammonia were used to recover light $CaCO_3$ from the mother liquor from brine salt production (Tang *et al.*, 1993).

Waste petroleum asphaltic acid, which contains sulfuric acid, has been neutralised with Na_2CO_3 to give Na_2SO_4 and the liberated CO_2 was used to carbonate a $Ca(OH)_2$ solution (Stejaru *et al.*,1978). Wastes from the production of dicyandiamide have been separated and fired at a temperature higher than the ignition point of free C but lower than the decomposition point of $CaCO_3$. The resulting $CaCO_3$ had a high purity (Tu, 1995).

Weak white liquor from the Kraft process was mixed with alkali (Na_2CO_3-Na_2S), filtered, causticized with $Ca(OH)_2$ and $CaCO_3$ sufficiently white for paper coating was precipitated. The filtrate was recycled (Raoux *et al.*, 1984). Green liquor from the pulp cooking process can also be used to recover $CaCO_3$ with excellent whiteness if air is first blown through the liquor to coagulate the dispersed carbon particles (Kumasaka and Otsuka,1986; Oishi *et al.*, 1989). Spent $Ca(OCl)_2$ bleaching liquors from pulp manufacture have also been treated to produce $CaCO_3$ and NH_4Cl of high purity (Khorasani *et al.*, 1983). Causticization muds in pulp mills have been washed with water and then carbonated with biogas. The $CaCO_3$ produced is used in the paper and has been shown to give the paper characteristics comparable to those when natural $CaCO_3$ is used. In addition, the biogas is upgraded by the utilisation (and removal) of the CO_2 (Perez *et al.*, 1990).

Calcium-containing ashes of residues produced in the paper industry were suspended in water and the filtrate was carbonated to recover $CaCO_3$ (Murr, 1997). Ash or impure calcium sources can also be acidified, filtered, brought to a pH above 6 to precipitate

impurities, filtered and carbonated with Na_2CO_3, $NaHCO_3$ or $(NH_4)_2CO_3$ (Szep *et al.* 1993; Blomquist and Betz, 1998).

Just as sugar solutions were used in the extraction of $CaCO_3$ from dolomite, so too has sugar been used to obtain higher calcium concentrations in solutions for carbonation (Belov and Valiullin, 1969; Saenz and Fernandez, 1991, Yamada *et al.*, 1991). Calcium solutions from a sugar factory have been carbonated using CO_2 from a distillery producing alcohol by fermentation to produce high purity $CaCO_3$ on an industrial scale (Rao and Gani, 1971).

1.2.7 Other Methods of Manufacture

The degassing of calcium bicarbonate solutions results in the formation of $CaCO_3$. This can be done using heat but ultrasonic vibrations have been shown to give better results (Cyrès, 1957). The addition of lime to hard water produces $CaCO_3$ which has a whiteness of 92 - 96 % as compared to MgO (Elek *et al.*, 1988; Woelfel, 1991). In one application, H_2CO_3 was produced from limestone, water and CO_2 and this was converted to $CaCO_3$ through the addition of $Ca(OH)_2$ (Yan, 1995).

Salt-rich water from thermal power plants has been treated to produce $CaCO_3$ which was sold to offset the costs and total water consumption (Nafdey *et al.*, 1981). Similarly, treatment of the waste water from the regeneration of cation-exchangers involves neutralisation with $Ca(OH)_2$ and the calcium can be recovered as $CaCO_3$ through the addition of soda ash (Nagpur and Sangal, 1988). Even water from geothermal vanes has been treated for the recovery of $CaCO_3$ and other compounds (Akhmedov *et al.*, 1991). $CaCO_3$ is also produced as a by-product in the manufacture of KNO_3 (Sun *et al.*, 1992).

Less usual sources of $CaCO_3$ have been used. There are patents covering the purification of $CaCO_3$ from egg shells (Contesso, 1947; Forbat, 1958; Maeda, 1982), sea shells (Bhatt *et al.*, 1990), oyster shells (Shearon, 1951; Shirosaka, 1983, Komatsu and Tanaka, 1987),

clam shells (Nelson *et al.*,1966) and waste crab exoskeleton (Muralidhara and Maggin, 1985). $CaCO_3$ purified from these sources is often used in preparations for human or animal consumption.

The aluminium content of a $CaCO_3$ intended for human consumption is important in terms of regulations for the prevention of Alzheimers disease. Al-containing CaO can be treated by dissolving the lime in HCl, separating the filtrate from the aluminium containing residue and carbonating the filtrate (Bacardi, 1995). Pharmaceutical grade $CaCO_3$ has also been prepared by carbonation of a calcium sucrose suspension (Mladenov *et al.*, 1972) and by reaction of CaO with aqueous ethanolammonium nitrate (or equimolar amounts of nitric acid and ethanolamine) to form $Ca(NO_3)_2$. The solution had a pH of 11, thus ensuring precipitation of all metallic impurities and after filtration and carbonation of the filtrate, the washed precipitate was greater that 99.9 % pure (Langelin, 1979; Langelin *et al.*, 1983). $CaCO_3$ used in the manufacture of toothpaste, was produced by treating an aqueous suspension of $CaSO_4$ from the decomposition of calcium citrate with a predetermined amount of aqueous K_2CO_3 or Na_2CO_3 solution (Hudec and Liska, 1985). The $CaSO_4$ can also be prepared from the reaction of a lime suspension with H_2SO_4 (Valiullin *et al.*, 1991).

1.3 Metal Removal

Many of the procedures outlined above involve precipitation of calcium carbonate from solutions containing metal ion impurities. These impurities have been removed by precipitation as insoluble hydroxide compounds but this is not the only option available.

1.3.1 Hydroxide Treatment versus Sulfide Treatment

Treatment of waters containing heavy metals with hydroxide is effective (giving almost complete precipitation), low-cost and convenient, especially in that automatic pH control is possible. A hydroxide salt is added to elevate the pH and metal hydroxides form. These

have very low solubilities at high pH (8 - 11) and settle out of solution over time periods of 2 - 4 hours. The process does have some limitations though:

- Hydroxide precipitates of amphoteric elements have a pH at which optimal precipitation is achieved. If the solution pH is changed in either direction, the precipitate tends to resolubilise.
- This means that removal of metals from solutions of mixed metal wastes may not be effective because minimal solubilities of individual elements occur at different pH values. In some cases, a compromise is made so that sufficient concentrations of each metal are precipitated or a series of precipitations is performed and metals are removed sequentially at their optimal pH.
- Chromium (VI) is not removed by hydroxide precipitation
- Cyanide interferes with heavy metal removal by hydroxide precipitation
- The presence of complexing agents may have an adverse effect on removal
- Hydroxide sludges have an amorphous particle structure and are generally voluminous and difficult to dewater
- Metal hydroxide sludges are often classified as hazardous wastes and must be disposed of in a secure landfill or engineered cell. The volume of the sludge becomes a financial consideration.

Sulfide precipitation has been used as a complement to hydroxide precipitation (Boling and Kobylinski, 1992) or as an alternative. The sulfide is either added in a soluble form, such as sodium sulfide (Na_2S) or sodium hydro-sulfide (NaHS), or an insoluble form, such as FeS. In the latter case, the sulfide salt dissolves to a certain extent, it reacts with metal ions and precipitates out of solution and to maintain the equilibrium concentration of sulfide ions, more sulfide salt dissolves. Sulfide precipitation has a number of advantages over hydroxide precipitation:

- There is a high degree of metal removal even at low pH (pH 2 - 3)
- Sulfides are highly reactive and reactor detention time is therefore low

- Selective metal removal and recovery is feasible
- Metal sulfide sludges exhibit better thickening and dewatering characteristics than the corresponding hydroxide sludges
- Metal sulfide sludges are three times less subject to leaching at pH 5, resulting in easier and safer disposal

There are concerns associated with sulfide treatment as sulfide is toxic and if an excess of sulfide reagent is added, the potential exists for hydrogen sulfide gas formation and escape. Measures can be taken to minimise this risk (Peters and Ku, 1985). The other disadvantage to this technique is the fact that there is a cost involved in the purchase of the sulfide reagents. A ready supply of cheap sulfide would be a distinct advantage.

1.3.2 Biological Sulfide

Sulfide can be produced by bacteria through the reduction of sulfate. Sulfide precipitation using a bacterial system has all of the advantages of sulfide precipitation mentioned above and in addition, it has been found that metal ions, even those that do not form sulfides, adsorb onto the bacterially produced FeS particles and are in this way removed from the liquid part of the system. The structure of bacterially produced iron sulfide has variable composition and behaves as a phase, rather than as a stoichiometric compound and has a large specific area, comparing well with that of activated charcoal (Watson *et al.*, 1995). The FeS produced also has variable magnetic properties and this can be used as a means to recover the precipitate (Watson *et al.*, 1996)

Utilisation of a bacterial system would not only accomplish metal removal but would also remove sulfate impurities, which would be necessary in the production of high-purity calcium carbonate.

1.4 Sulfate Reducing Bacteria

Sulfate reducing bacteria (SRB) are bacteria that use sulfate ions (SO_4^{2-}) as electron acceptors in the oxidation of a substrate and produce sulfide ions (S^{2-}). This process is carried out by a diverse heterogeneous group of obligately anaerobic eubacteria, the majority of which are mesophilic, although there are some important thermophiles (Hansen, 1988).

1.4.1 Morphology and Physiology of SRB

SRB are usually identified by the prefix "Desulfo" in the genus name. The remainder of the genus name and the species nomenclature shows the diversity of this group. Classification is based primarily on nutritional and morphological characteristics but is complicated by the fact that morphologically similar types may differ in their nutrition and nutritionally similar types may differ in their morphology. Morphologically, SRB may be rods (like *Desulfomaculum nigrificans*), some of which are distinctly curved (like *Desulfomaculum acetoxidans*), vibrios (like *Desulfovibrio desulfuricans*) or spheres (like *Desulfococcus multivorans*) and may also take intermediary forms such as oval or lemon-shaped cells (one example of which is *Desulfobacter postgatei*).

The growth requirements of different SRB species are generally similar. The optimal temperature is usually around 30°C, except for the thermophiles; optimal pH is usually 7 (although SRB can grow between pH 6 and 9) and anaerobic conditions are required. This said, SRB often prove resilient in the face of unfavourable conditions. Although *Desulfobacter hydrogenophilus* has an optimal temperature of 32°C, slow growth at 0°C has been observed (Widdel, 1988). Recently acidophilic SRB have been identified in acid mine drainage waste waters (Johsen, 1998) and in aerobic environments such as well-aerated activated sewage sludge (Widdel, 1988). This is often made possible by the formation of favourable microniches. In sediment pellets, for example, the oxygen can be depleted and the pH raised (through production of hydrogen ion scavengers HS^- and

HCO_3^-) to enable growth. SRB are obligate anaerobes and while some are able to live without sulfate present, they are usually found in habitats where dissolved sulfate is available (Widdel, 1988).

1.4.2 Heterotrophic Growth

Chemically, the reduction of sulfate must be coupled with the oxidation of another substrate. There are two major metabolic groups: those that oxidise the substrate incompletely to acetate and those capable of complete oxidation to carbon dioxide. The first group commonly utilise lactate, pyruvate, malate, fumarate or ethanol and are often also able to utilise hydrogen as sole energy source. Incomplete oxidisers are usually fast growing species. Complete oxidisers can, in general, oxidise a greater variety of organic carbon sources but tend to have longer doubling times (Widdel, 1988).

Most natural waters contain sulfate and some organic compounds. The degradation of the organic material depletes the dissolved oxygen in the water, allowing the growth of anaerobic bacteria which utilise low-molecular weight compounds, such as the products of bacterial fermentation of the available organic matter. In these environments, however, there is competition for the available nutrients. SRB capable of growing on hydrogen and acetate compete with methanogens which convert the same nutrients into methane. Fermentable compounds such as lactate and ethanol are also used by fermentative bacteria, adding another dimension to the competition (Widdel, 1988).

It has been found that the presence of non-limiting sulfate inhibits the growth of methanogens and through control of the ratio of sulfate concentration to chemical oxygen demand, cultures can be enriched for SRB (Harada *et al.*, 1994). Methanogens and SRB compete for available H_2 and acetate. Thermodynamically, the free energy change for acetate and H_2 utilisation by SRB reactions is greater than that for methanogens reactions thus giving SRB the competitive advantage under standard conditions. Kinetically, SRB also have the competitive edge but the advantage is only slight and insignificant in

practical terms. When the electron donor:sulfate ratios become high or when build-up of sulfide occurs, this situation can be reversed. The specific ratio depends on the nutrient source but in general, a ratio below 1 should give SRB the advantage (McCartney and Oleszkiewicz, 1993). Anaerobic mesophilic enrichments with organic acids, ethanol (or higher alcohols) or complex organic material in the presence of sulfate, usually select for SRB as the terminal degraders. Significant methane production occurs when the temperature is raised above 55°C or if the culture is kept in flocculated sludge digesters. The rationale behind the latter is that SRB attach less tightly to matter than methanogens so although the SRB are active, their overall influence is limited by the loss of SRB by washout (Widdel, 1988)

1.4.3 Autotrophic Growth

The feasibility of growing SRB autotrophically has been the focus of much research. Growth using carbon dioxide as sole carbon source, hydrogen as energy source and electron donor and sulfate as the electron acceptor is possible for *Desulfosarcina variabilis* (Pfennig *et al.*, 1981), *Desulfonema limicola* (Widdel *et al.*, 1983), *Desulfococcus niacini*, (Imhoff-Stuckle and Pfennig, 1983) and *Desulfotomaculum orientis* (Klemps *et al*, 1985; Cypionka, 1986; Cypionka and Pfennig, 1986). *Desulfovibrio* strains were shown to be chemolithoheterotrophic. Growth on hydrogen and carbon dioxide was only possible in the presence of some simple carbon sources (acetate in this case) which provided two-thirds of the carbon for assimilation to cell material (Badziong *et al.*, 1978; Brandis and Thauer, 1981).

Enrichment of natural samples with hydrogen in liquid mineral media, mostly yielded mixed cultures of *Desulfovibrio* and autotrophic homoacetogens of the *Acetobacterium* type that produced acetate that was in turn utilised by the sulfate reducers. These mixed cultures can simulate cultures of autotrophic sulfate reducers, especially as the *Acetobacterium* are found in such low numbers in comparson to the *Desulfovibrio*

(Widdel, 1988). SRB commonly use ammonium ions as a nitrogen source although those capable of reducing nitrate to ammonia may be able to use nitrate ions (Widdel, 1988).

1.4.4 Sulfide Production

SRB produce sulfide ions. These ions (as well as hydrogen sulfide which is often formed from the sulfide ions) are toxic to living organisms even in small amounts. The fate of the sulfide is therefore of importance. Sulfide is eliminated from natural systems in a number of ways. Firstly, sulfide that comes into contact with an oxic environment, may be oxidised chemically to sulfur, sulfite or thiosulfate. Sulfide oxidation is also performed by aerobic colourless sulfur bacteria that convert the sulfide to sulfur or sulfate. In the anaerobic region, sulfide oxidation is carried out by phototrophic sulfide-oxidising bacteria. These produce sulfate ultimately, with sulfur as a significant intermediate and complete the natural sulfur cycle (Figure 1.3).

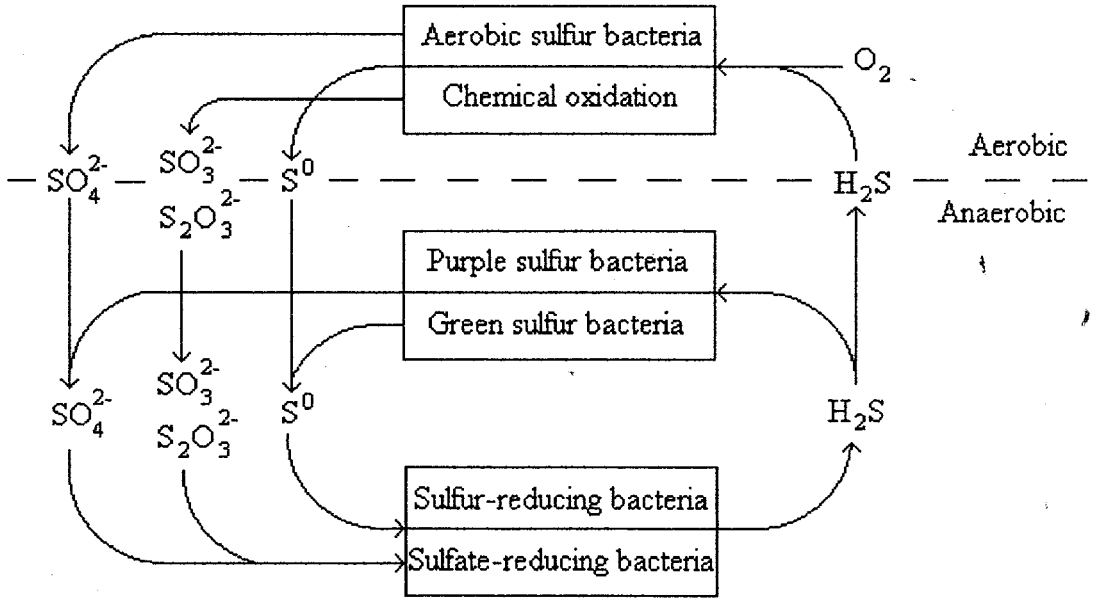

Figure 1.3 The natural sulfur cycle (Widdel, 1988)

Sulfide is also a chemically reactive ion and readily forms metal sulfides with metals present in the environment. As previously mentioned, these are very sparingly soluble and

precipitate out of the solution (Widdel, 1988). It is this reaction that has caught the attention of industry and has transformed the SRB into a biotechnological asset.

1.4.5 Heterotrophic Production of Sulfide

It has been know for many decades that sulfate reducing bacteria can live in the acidic water that seeps from mines (acid mine drainage). Studies have been made on natural ecosystems that treat acid mine drainage. In one example, the seepage from the mine flows through a pile of wood dust where, through the degradative action of micro-organisms on the cellulose, it acquires a simple carbon source. This supports growth of SRB in the pond following the wood pile. In this pond, heavy metal contaminants are precipitated as sulfides, the sulfate concentration is decreased and the pH of the water is raised (Tuttle *et al.*, 1969). This discovery has been further investigated to determine the effect of additions of specific organic nutrients. Wheat bran, glucose, peptone and the sodium salts of formate, pyruvate and lactate caused a significant increase in sulfate reduction and pH elevation. The last three additions are substrates for SRB and the first three would have been degraded by other heterotrophic bacteria in the medium to give suitable substrates (Wakao *et al.*, 1979).

Application of this natural process has been used by some industries. In an artificial application, the SRB need a source of simple carbon compounds. Some processes have used ethanol as the energy source for the bacteria as it is a readily available chemical and was cheaper in this application than lactate. In this case, nitrogen and phosphorus compounds were also added as nutrients (Barnes *et al.*, 1991). Mixed bacterial cultures are often used in industrial systems as the mixed population is able to adapt to minor changes in conditions each of the populations may have different optimal conditions. Utilisation of a mixed culture also means that more complex carbon sources can be added as nutrients as these can be degraded to compounds that SRB can use (White and Gadd, 1996). Whey has also been used as a source of organic nutrients. In this case, fermentative bacteria were needed to degrade the lactose to simpler compounds

(Christensen *et al.*, 1996). Lactate and ammonium chloride (as a nitrogen source) have been used as growth nutrients (Wijaya *et al.*, 1993).

Molasses has been used successfully as a nutrient but in this case, it was found that the bacterial support was important for growth. It was proposed that the bacteria obtained trace elements from a stone support which they could not obtain from sand or plastic and this resulted in better growth (Maree, 1984; Maree *et al.*, 1991). Press-juice is another feedstock which has been used with success (Riekkola-Vanhanen and Mustikkamaki, 1997) Cattle waste has been used as a cheap source of SRB and nutrients. Experiments found that nutrients were not limiting, allowing growth of both SRB and methanogens resulting in production of methane as well as precipitation of metals. H_2 was found to be a limiting nutrient as both populations made use of it and this can be used as a tool to enhance the activity of the SRB (Ueki *et al.*, 1991). Straw has also been tested as a cheap nutrient. This substrate does however necessitate a long retention time to allow its breakdown into simple compounds used by the SRB (Béchard *et al.*, 1993).

1.4.6 Autotrophic Production of Sulfide

A number of systems have been developed to utilise simple inorganic compounds for the growth of the SRB. Although the addition of growth supplements greatly stimulated bacterial growth SRB mixed cultures (inoculum from sewage) could grow and reduce sulfate using producer gas as the sole carbon and energy source (Dill *et al.*, 1995). Producer gas is a mixture of CO, CO_2, H_2 and N_2 and is cheaply available from certain industrial processes such as from steam and methane, by the partial oxidation of fuel oil or the gasification of coal. The use of producer gas as opposed to the addition of an organic carbon source is attractive because: there is no need to remove the organic products or by-products after treatment (there will be no excess organic matter in the discharged water); it is widely available as a by-product from coal-burners; and low-grade, sulfur-containing coal can be used as the sulfur compounds will be treated by the system (Du

Preez *et al.*, 1992). Biologically, the addition of H_2 instead of an organic carbon source is advantageous as SRB out-compete methanogens under these growth conditions.

For producer gas to be used by the SRB, the CO must first be converted substrates that the SRB can use. CO inhibits growth of SRB (Karpilova *et al.*, 1983, Mukhitova *et al.*, 1983). Certain species of SRB were able to grow in the presence of limited amounts of CO and this growth was accompanied by the production of H_2 (Lupton *et al.*, 1984). This correlates with the work of other researchers who found that certain SRB could convert CO to CO_2 and H_2 through the use of a water shift (Karpilova *et al.*, 1983; Yagi, 1958; Yagi and Tamiyia, 1962):

$$CO + H_2O \rightarrow H_2 + CO_2$$

In a mixed culture, this can also be achieved by non-sulfate reducing micro-organisms such as *Methanosarcina barkerii* (Kluyver and Schnellen, 1947); symbiotic purple non-sulfur bacteria like *Rhodopseudomonas gelatinosa* (Du Preez *et al.*, 1992); photosynthetic bacteria like *Rhodospirrilum rubrum* (Klasson *et al.*, 1990) or mixed culture anaerobes (Levy *et al.*, 1981). Another option is to use homo-acetogenic bacteria such as *Acetobacterium* or *Clostridium* species to reduce two molecules of CO or CO_2 to acetate. Increased acetate concentrations do encourage active competition between SRB and methanogens however, leading to complex interactions. In a study in which the carbon component of the producer gas composition was gradually changed from CO_2 to CO, the sulfate conversion rate was seen to drop gradually and at a microscopic level, changes in the biological layout were seen. Instead of growing on the support (pumice particles), the SRB grew in aggregates with *Acetobacterium* species, the latter growing on the outside of the aggregates (Van Houten, 1996).

It was also found that sulfate reduction using only CO as an energy source is possible. The suggestion is that the SRB use the reaction (Du Preez and Maree, 1994):

$$4\ CO + SO_4^{2-+} \rightarrow S^{2-} + 4\ CO_2$$

Desulfovibrio desulfuricans can carry out this reaction (Yagi and Tamiya, 1962)

1.4.7 Reactors

Some applications of the above processes involve adding nutrients to an existing mine site which has filled with water and is starting to overflow. The advantage of this is the ease of treatment but control of the effluent is made difficult. The effluent may still be toxic or may contain excess nutrients and facilitate other microbial growth (Christensen *et al.*, 1996).

More control is brought to the system if the drainage water is removed from the source and treated in a reactor. The acid mine drainage is directed through a bioreactor which is supplied with a source of nutrients for the SRB. The sulfate is reduced to sulfide which reacts with the metals in the solution to form an insoluble precipitate. The effluent water then has decreased concentrations of metal ions and sulfate. In these systems, the flow rate must be controlled to ensure that the pH does not drop too low for survival of the SRB and there are fluctuation in the mine water which translate to the column, changing the growth conditions. The metals are precipitated in the bioreactor and may cause plugging, abrasion and toxicity and metals are precipitated together, making extraction of useful metals difficult. The final sludge also contains biomass which contributes to the volume of sludge to be disposed.

Another option is the Biosulfide process in which the chemical precipitation of metals with sulfide is separated from the biological conversion of sulfate to sulfide (Rowley *et al.*, 1994). The acid mine drainage is directed through a number of precipitation vessels and in each vessel, the pH is adjusted through the addition of water from the biological circuit. This water contains sulfide and alkalinity in the form of carbonate from the reduction of sulfate by SRB. The pH is controlled to give the optimal pH of precipitation of a specific

metal sulfide in each vessel. This allows selective recovery of metal concentrates (for resale to smelters) and the remaining metals are precipitated in the last vessel to give a metal sludge for disposal. The treated mine water is directed into the biological circuit as a sulfate source. Ultimately, the biomass can also be removed and sold. Practical optimisation of such a system is important as the theoretical optimal pH of precipitation of a metal sulfide may differ from the pH at which it actually does precipitate in a mixed metal solution. The flow rate of the mine water is also important to ensure that the precipitate has enough time to settle and is not carried over in to the next precipitation vessel (Rowley *et al.*, 1994).

One drawback of the above system was the formation of sulfur in the first vessel by reaction with ferric ions:

$$S^{2-} + 2\ Fe^{3+} \rightarrow S^{0} + 2\ Fe^{2+}$$

This can be avoided, in some cases, by using "fresh" acid mine drainage in which the ferrous ions have not been converted to ferric ions (Rowley *et al.*, 1994). Alternatively, a preliminary bioreactor containing heterotrophic, iron-reducing, acidophilic bacteria immobilised on an organic support matrix could be added. These bacteria decrease the redox potential of the mine water by reducing ferric ions to ferrous ions and their use of the organic support matrix as a nutrient source adds small molecular weight organic molecules to the medium for use by the SRB (Johnson, 1995).

It must be remembered that in the above system, there may be sulfide left in the effluent water after precipitation of all metals. This sulfide is toxic and should not be discharged into the environment. It has been proposed that water emerging from the reduction vessel be directed into a biological oxidation vessel. Here sulfide oxidising bacteria convert it to solid sulfur, which can be sold to offset costs (Herrera *et al.*, 1991) Bacteria responsible for this include the green and purple sulfur bacteria, *Chlorobium* and *Chromatium* species. Some excrete the sulfur into the process water, *Chlorobium thiosulfatophilum* excretes colloidal sulfur and *Chlorobium finosum* stores sulfur intracellularly (Maree *et al.*, 1986).

A biological oxidation step is also useful in lowering the COD content of the effluent water and residual metals, notably iron and manganese may even be precipitated in this stage by biological oxidation to insoluble forms. The chemical option would be to add a source of ferric ions to precipitate the sulfide as ferric sulfide (Maree *et al.*, 1987a and b).

1.4.8 Biological Reduction of Sulphate under Alkaline Conditions

While much work has been done on sulfate reduction in acidic conditions, there is little mention of the activity of sulfate reducing bacteria in alkaline environments. In this investigation, solutions of all of the starting materials studied were neutral or basic and evidence of the ability of SRB to reduce sulfate in alkaline solutions was therefore important. Sulfate reduction has been observed in Icelandic hot springs with a pH of 8.7 (Sonne-Hansen and Ahring, 1997). SRB are inhibited at pH values above 9 although it is possible that they may be able to grow in microniches at higher pH's in the same way that they are able to survive low pH conditions (Widdel, 1988).

1.5 Manufacture of Calcium Carbonate Using Biological Systems

A few processes for the manufacture of $CaCO_3$ make use of biological systems. These usually use sulfate reducing bacteria to convert gypsum into elemental sulfur and $CaCO_3$.

Coal burning at power plants produces sulfur dioxide. When discharged into the environment, this causes acid rain and the gas is therefore treated with limestone before it is released. The result is a calcium sulfate and sulfite waste know as flue gas desulfurisation (FGD) gypsum. Although FGD gypsum it can be used in wallboard or cement, often this is not economically feasible or the impurities in the gypsum limit applications and the FGD gypsum must be disposed of. Regulations do not always permit simple landfill of this waste and costs of disposal can therefore be high. Cheap recycling of this material is very attractive (Kaufman *et al*, 1996).

If biological systems are to be used to achieve this goal, the choice of nutrient source for the sulfate reducing bacteria (SRB) becomes important as pure chemical feeds (such as ethanol or lactate) make the reduction too expensive (Apel and Barnes, 1993). Molasses is less expensive but is incompletely digested in a single anaerobic reactor. When an aerobic vessel and another anaerobic vessel follow the initial SRB reactor, the effluent water has good relative purity (Maree and Hill, 1989). Coupling of an autotrophic system to the heterotrophic SRB system may improve the feasibility but not if the photosynthetic bacteria (green sulfur bacteria, *Chlorobium*, in this case) require high intensity electric lights in order to produce the organic acids required by the SRB (Kaufman *et al.*, 1996). A Belgian patent describes a process whereby the SRB were fed a mixture of acetic acid, calcium sulfate and sulfite wastes and organic waste from a swine farm and the H_2S produced was chemically converted to sulfur (Olthof *et al.*, 1984).

Economic analyses have been done and these concluded that the fixed capital investment costs for a biological or conventional hydrotreatment plant were identical. The difference was seen in the choice of carbon source – corn hydrolyzate was too expensive but anaerobically digested municipal sewage sludge may be feasible (Sublette and Gwozdz, 1991; Selvaraj and Sublette, 1996). The latter system has been investigated and found successful. SRB were immobilised on BIO-SEP™ beads in a column and fed sewage sludge and FGD gypsum. Total reduction of the sulfate was achieved and 70 % was recovered as sulfur by oxidation with ferric sulfate. The ferrous sulfate generated is converted to ferric sulfate by a *Thiobacillus* culture. The spent solution was made alkaline by the addition of NaOH and was carbonated to give $CaCO_3$ which was recycled (Kaufman *et al.*, 1996).

1.6 The South African Context

White lime is not presently manufactured in South Africa. Previous work has shown that many methods for this manufacture are available but not all are suitable for application in

South Africa. What is needed is an inexpensive, simple system that will produce large quantities of white limestone.

Natural limestone beneficiation processes may either not give a sufficiently pure $CaCO_3$ or make use of chemicals which increase the cost of manufacture. Carbonation of a lime suspension does not guarantee complete conversion to $CaCO_3$ and where unreacted particles are found in the product, it is likely that impurities will also be found. Carbonation of a calcium hydroxide solution certainly fulfils the requirement for simplicity but the low solubility translates into low yields. The applications discussed in this chapter were more suited to the production of small quantities of $CaCO_3$. Utilisation of chemicals that improve the solubility of lime in water adds to the cost. Where the solutions are recycled, the running cost is not greatly elevated but the initial cost is high and chemicals will need to be replenished or replaced. The capital cost is also a consideration. Ammonia and ammonium salts and acids of various kinds are not inert substances and construction of plants in which they are used must reflect this.

Preparation of $CaCO_3$ from pure chemicals, while producing a product of high purity, is prohibitively expensive for industrial scale manufacture. When the source of these chemicals is a waste stream from another industry, the cost is lowered but as the main lime factories in South Africa are located in the Northern Cape, many kilometres from other industries, the cost of transporting waste materials to the factories (which is the source of carbon dioxide for many of the processes) is expensive. In addition, these products may not be sufficiently pure and further treatment may be necessary.

1.7 Research Objectives

The problems mentioned above are not insurmountable and many procedures may potentially work. Financial and practical evaluation of these methods may well show that white lime could be produced using an existing technology, at sufficiently low cost and high purity to be feasible. However, the need for an inexpensive, simple, large-scale process still remains and there is scope for more work to be done in this area. In addition, there is a market for $CaCO_3$ that is not only white (free of coloured impurities) but is also chemically pure. The research objectives of this study were focused on the above and were identified as follows:

1) To investigate the chemistry of heavy metal removal from lime
2) To determine the purity of calcium carbonate prepared from the starting materials available at Lime Acres
3) To determine whether or not sulfate reducing bacteria can grow and reduce sulfate under the conditions relevant to this study
4) To design a biotechnological process for the production of calcium carbonate utilising the activity of sulfate reducing bacteria

Chapter 2: Hydroxide and Sulfide Precipitation Studies

2.1 Introduction

Natural limestone contains not only calcium carbonate but also traces of other minerals. Minerals containing transition metal elements have the potential to impart colour to the limestone and limestone containing these is not white calcium carbonate but rather grey or brown in colour. The main transition metals found in limestone mined at Lime Acres are manganese, iron and titanium. Titanium oxide is white while oxides of manganese and iron are brown/red (Cotton *et al.*, 1987). Therefore, we sought to remove iron and manganese from the starting material.

Before embarking on an investigation of the potential for using biological systems to remove metals from a solution, it was first necessary to investigate briefly their chemical removal. This is most commonly achieved through precipitation with hydroxide or sulfide ions (Peters and Ku, 1985).

Most manganese(II) salts in neutral or acidic media, are water soluble. Addition of hydroxide ions (OH^-), initially produces a gelatinous white hydroxide which rapidly oxidises to Mn_2O_3 with a corresponding darkening of the colour. Manganese dioxide (MnO_2) is the final oxidation product and this has a grey to black colour. The addition of HS^- initially gives hydrous MnS but this also oxides with time giving the same products as before.

The addition of OH^- to solutions of iron(II) initially produces the green hydroxide but oxidation continues to give the red-brown hydrous iron(III) oxide ($Fe_2O_3.nH_2O$) (Cotton *et al.*, 1987). Addition of HS^- results in an amorphous FeS precipitate which is converted to more stable iron sulfides with time (Béchard *et al.*, 1993).

The solubility products of the metals of interest are shown in Table 2.1.

Table 2.1 Solubility products of the hydroxide and sulfide compounds of selected metals (Callander and Barford, 1983)

	K_{SP}	
Metal	OH^-	S^{2-}
Ca^{2+}	4×10^{-11}	Soluble
Mn^{2+}	7×10^{-15}	6×10^{-16}
Fe^{2+}	2×10^{-15}	1×10^{-18}

From this information it should be possible to determine the concentrations of metals remaining in solutions with varying concentrations of OH^- or S^{2-}. The relevant equation is (Atkins *et al.*, 1992):

$$K_{SP} = [M^{2+}][OH^-] \text{ or } K_{SP} = [M^{2+}][S^{2-}]$$

It has been found though, that theoretical predictions based on data for single metal ion solutions seldom correlate well with the actual situation in solutions containing multiple metal ions (Callander and Barford, 1983).

For this reason, it is necessary to evaluate the precipitation experimentally whenever a new system is studied. The precipitation chemistry of calcium hydroxide produced at Lime Acres was studied in two experiments. A study of the precipitation of manganese and iron with hydroxide was followed by a study of their precipitation with sulfide ions.

2.2 Precipitation with Hydroxide

An investigation into hydroxide precipitation of manganese and iron from a solution of Lime Acres slaked lime.

2.2.1 Materials and Methods

$Ca(OH)_2$ (10 g) was completely dissolved in 33 ml concentrated HCl and made up to a volume of 100 ml using milli-Q water (Millipore). The pH of the solution was measured and subsequently raised to set values (3.8; 5.2; 6.4; 7.2 and 8.25) using 20 % NaOH and a precipitate formed. The suspension was filtered. The pH of the filtrate was adjusted to 10.3 using 20 % NaOH. CO_2 gas was bubbled through the solution until the pH was approximately 8. The suspension was filtered.

2.2.1.1 Sampling

Samples were taken according to the following scheme:

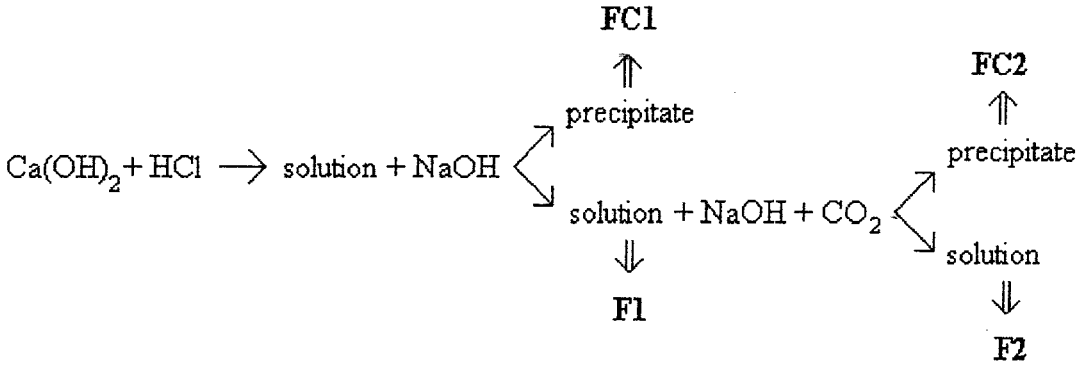

Figure 2.1 Sampling scheme for hydroxide precipitation

FC1 refers to the filter cake from the first precipitation

F1 refers to the filtrate from the first precipitation

F2 refers to the filtrate from the second precipitation

FC2 refers to the filter cake from the second precipitation

2.2.1.2 Chemical Analysis

pH measurements were made using a calibrated Cyberscan 2000 pH meter.

Liquid samples were diluted where necessary and analysed for Ca, Mn and Fe using a GBC 909 AA atomic absorption spectrophotometer. Solid samples were dissolved in HCl, made up to a standard volume and analysed using atomic absorption spectrophotometry.

2.2.2 Results and Discussion

2.2.2.1 pH analysis

The pH of the initial solution was 0. It was necessary to drop the pH to this value in order to keep the iron and manganese in solution as iron(III) hydrolyses at pH values higher than 2 (Cotton *et al.*, 1987).

2.2.2.2 Atomic Absorption Analysis

The results of the atomic absorption analyses are shown in Tables 2.2 and 2.3. The abbreviations introduced in the sampling scheme have been used in these tables.

Table 2.2 Manganese and iron levels in samples taken during hydroxide precipitation (measured in mg/10g original sample)

	Manganese				Iron			
pH	FC1	F1	F2	FC2	FC1	F1	F2	FC2
3.8	12.341	43.598	13.552	18.910	15.235	0.226	0.163	0.081
5.2	4.032	47.195	28.705	14.262	0.448	0.218	0.179	0.025
6.5	12.044	42.090	28.923	8.760	0.119	0.135	0.071	0.049
7.2	25.784	14.852	1.427	7.901	0.155	0.085	0.054	0.019
8.25	9.589	7.642	6.804	10.375	14.487	0.305	0.241	0.041

Table 2.3 Calcium levels in samples taken during hydroxide precipitation (measured in g/10g original sample)

pH	FC1	F1	F2	FC2
3.8	0.075	6.060	4.517	0.276
5.2	0.009	4.982	5.116	0.119
6.5	0.029	5.595	4.096	0.370
7.2	0.340	4.067	5.564	0.054
8.25	0.028	3.427	2.118	0.038

The composition of the first filter cake showed the precipitation of the metals of interest at the pH values investigated. This is depicted in figure 2.2. The amount of calcium precipitated at these pH values is shown as well.

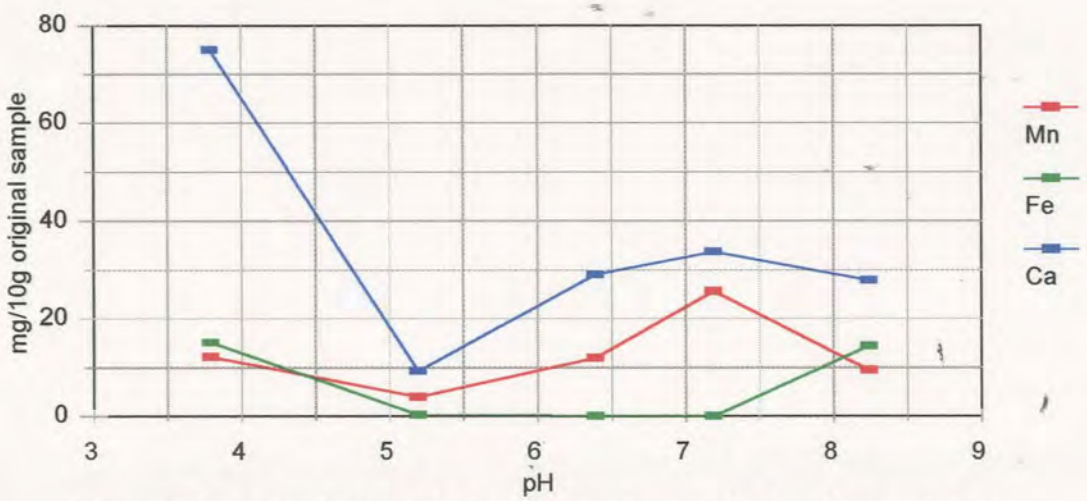

Figure 2.2 Analysis of the first filter cake after hydroxide precipitation

The second filter cake was the $CaCO_3$ precipitate and to be acceptable, should contain no manganese or iron while the calcium carbonate content should be maximised. The levels of iron and manganese found in this precipitate are shown in figure 2.3.

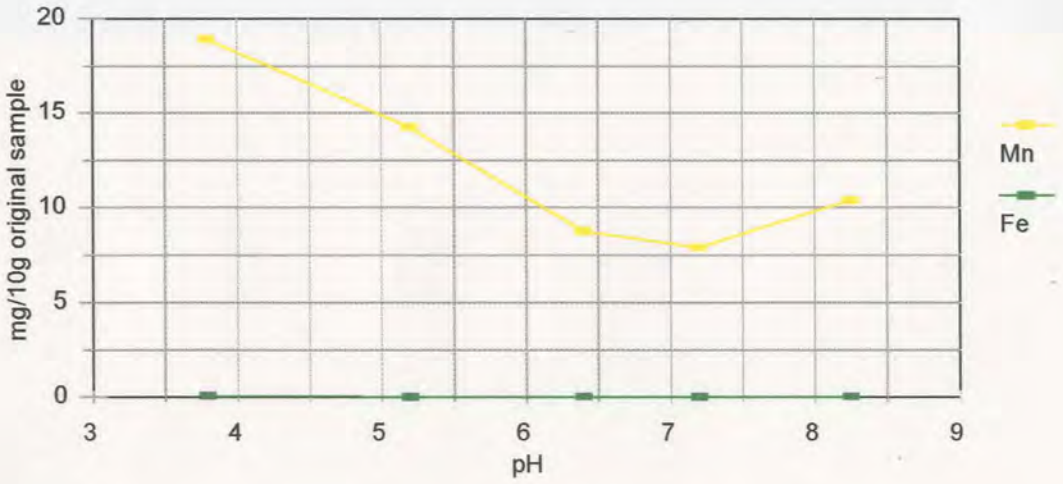

Figure 2.3 Analysis of the second filter cake after hydroxide precipitation and carbonation

Notes on the Experimental Procedure: The results shown in the graph did not give a very clear trend. It was found during the analysis, that samples prepared and assayed in the same batch (5.2, 6.5 and 7.2) gave comparable results but samples which were analysed separately (3.8 and 8.25) gave results which did not correlate well with other results. For the purposes of the current study, these results were sufficient as the aim was simply to acquire a better understanding of the metal interactions in the material and the feasibility of using hydroxide precipitation to remove all traces of metal. The numerical results here may not be accurate, but a useful trend was seen in the results of the second, third and fourth experiment.

If a rigorous analysis of the optimal pH of precipitation of iron and manganese in this material is required at some point in the future, it is recommended that a large mass of sample is dissolved and that aliquots of this are used for all of the pH studies. The samples should then all be analysed at the same time on the atomic absorption spectrophotometer and the same calibration curve should be used. The experiments should be performed in rapid succession to avoid discrepancies caused by changing precipitate composition with ageing and atmospheric oxidation.

The slaked lime sample was completely dissolved in acid to ensure that all metals in the solution were present in ionic form. HNO_3 was initially the acid chosen for the dissolution but it was found that not all components of the slaked lime dissolved in this medium. Fe_2O_3 is particularly resistant to dissolution although slow dissolution in cold HCl has been reported (Comey and Hahn, 1921). Using HCl complete dissolution of the calcium hydroxide, iron and manganese oxides was achieved.

The pH of the solution was then raised using sodium hydroxide and manganese and iron hydroxides began to precipitate from the solution. The amount of manganese and iron found in the precipitate increased as the pH of the initial precipitation was increased. This was expected as increasing pH corresponds to increasing concentrations of hydroxide ions available for reaction with the metals. Increasing amounts of calcium were also removed as sparingly soluble calcium hydroxide formed and precipitated out of solution.

Hydroxide Precipitation: The levels of iron and manganese in the second filter cake decreased as more of the metal ions were removed in the first precipitation. The amount of calcium fluctuated. This can be attributed to the fact that the calcium carbonate precipitation was not accurately controlled in terms of pH. As mentioned in the first chapter, careful monitoring of the progression of the carbonation is necessary to prevent conversion of insoluble calcium carbonate to soluble calcium bicarbonate (Kosin and Andrews, 1989; Takuma, 1991). In this case, the concentration of calcium in the filtrate was much higher than in the filter cake. At optimal pH conditions, this would not be the case and with more accurate control, complete precipitation in all samples could be ensured. This should give more stable results and better yields.

Iron Removal: The results showed that the levels of contaminating metals in the final product were gradually decreased by precipitation at increasingly high pH values. For treatment by hydroxide precipitation to be feasible, complete removal of the iron and manganese would be necessary but this was not achieved. In the case of iron, most of this metal was removed fairly early in the precipitation procedure and even at a pH of 3.8,

most was precipitated in the first filter cake and the first filtrate was almost free of iron. This is consistent with the literature which shows that iron(III) hydrolyses at pH values as low as between 2 and 3 to form iron hydroxides which precipitate as gels and finally hydrous Fe_2O_3 is formed (Cotton *et al.*, 1987). Traces of iron were still found in the final product, however, and this is not acceptable.

Manganese Removal: The manganese was even more difficult to remove using simple pH precipitation. Even at a pH of 8.25, a substantial portion of the manganese remained soluble. This was a clear indication of the fact that theoretical metal ion solubilities are not always directly applicable to real solutions containing multiple metal ions. The theoretical solubility of manganese hydroxide in a solution with a pH of 8.25 can be calculated:

$$pH = -\log[H^+]$$

When pH is 8.25,

$$[H^+] = 10^{-8.25}$$
$$= 5.62 \times 10^{-9}$$

$$10^{-14} = [OH^-][H^+]$$
$$[OH^-] = 10^{-14}/[H^+]$$
$$= 1.78 \times 10^{-6}$$

$$K_{SP} = [OH^-][Mn^{2+}]$$
$$[Mn^{2+}] = K_{SP}/[OH^-]$$
$$= 3.94 \times 10^{-9}$$

This is clearly a much lower concentration than was found experimentally. Some of the manganese which remained in solution after the initial precipitation, then precipitated out in the final $CaCO_3$. The precipitate had a pale brown colour, even when the 8.25 pH precipitation was used. This was clearly not pure enough for the purposes of producing white calcium carbonate.

It would have been possible to continue the pH studies, using higher pH values for the initial precipitation but the amount of manganese that remained in the solution for the pH values 6.5, 7.2 and 8.25 was not greatly different. It seemed that once the manganese had been solubilised, it did not easily re-precipitate as an insoluble hydroxide.

It must also be considered that the calcium content of the solution was also decreasing with each successive precipitation. It may be possible to remove all manganese through the use of a high pH initial precipitation but much calcium would also be lost in this step. This would decrease the final yield of calcium carbonate which is precisely what was to be avoided.

Practical Relevance: The problem of removing soluble metals is of immediate practical relevance if white calcium carbonate is to be manufactured using one of the methods (mentioned in Chapter 1) that first involve complete acid dissolution. Impurities like iron and manganese would be solubilised and if not removed, would precipitate as carbonates during the carbonation step, making the final product impure. This emphasises the necessity of the complicated purification procedures often part of such methods (Angelov and Khainson, 1956; Papageorgios and Lobacz, 1973; Gol'dinov *et al.*, 1976; Langelin, 1976; Smolik and Ogiolda, 1979, Karakhanyan *et al.*, 1983; Ryabukha *et al.*, 1985; 1986). Methods using sugar or ammonia solutions to increase the solubility of the calcium source would also face this problem as some manganese oxides are soluble in ammonia salts and both iron and manganese oxides are soluble in sucrate solutions (Comey and Hahn, 1921; Weast and Astle, 1981). If the source material does not contain iron and manganese, the ammonia and sucrate dissolution methods may work well but with the starting materials available at Lime Acres, it is unlikely that the product would be a sufficiently white calcium carbonate.

2.3 Precipitation with Sulfide

An investigation into the removal of manganese and iron from a slaked lime suspension using sulfide ions.

2.3.1 Materials and Methods

$Ca(OH)_2$ (10 g) was weighed into a bottle with a volume slightly larger than 100 ml and was fitted with a stopper. The bottle volume was chosen to minimise contact between the solution and atmospheric carbon dioxide and therefore minimise carbonation during the course of the experiment. 100 ml milli-Q water (Millipore) was added and the bottle was sealed. The suspension was shaken on a mechanical shaker for 10 minutes after which it was filtered using a vacuum pump. The filter cake was dried at 60°C and sealed in a polytop vial. Four experiments were performed using this general procedure with the following modifications:

- In the second experiment 0.2 M sodium sulfide (Na_2S) was used instead of milli-Q water in step 2
- In the third experiment, H_2S water (prepared by bubbling H_2S through a volume of water until the pH stabilised) was used instead of milli-Q water and because H_2S water has a low concentration of sulfide ions, the sample mass was decreased to 1 g instead of 10g
- In the fourth experiment H_2S was bubbled through the slaked lime suspension

2.3.1.1 Sampling

The filtrate and filter cake were analysed.

2.3.1.2 Chemical Analyses

A pressed bead was prepared from the dried, ground filter cake material (procedure given in Appendix A) and this was analysed using a Siemens MRS 400 MP X-Ray Fluorescence Spectrometer (XRF). A portion of the filter cake was dissolved in acid and analysed for Mn, Fe and Ca using atomic absorption spectroscopy.

The pH of the filtrate was measured and it was analysed for soluble sulfide using a spectrophotometric method (Appendix A). The filtrate was also analysed for Mn, Fe and Ca by atomic absorption spectroscopy.

2.3.2 Results and Discussion

2.3.2.1 Observations

In all cases, except when Na_2S was used, the filtrate was clear and colourless. When Na_2S was used, the filtrate was initially clear and yellow but the colour faded after two days. The colour of the filter cake was seen to change when different solutions were used. When milli-Q water was used, the colour of the filter cake was the same as that of the original sample. When any of the other solutions was used, the filter cake became progressively lighter in colour and black speckles were seen in the filter cake. The filter cake was also voluminous when the milli-Q water was used whereas when sulfide was present, the filter cake was more compact.

2.3.2.2 XRF analyses of Filter Cakes

The XRF data is shown in figure 2.4

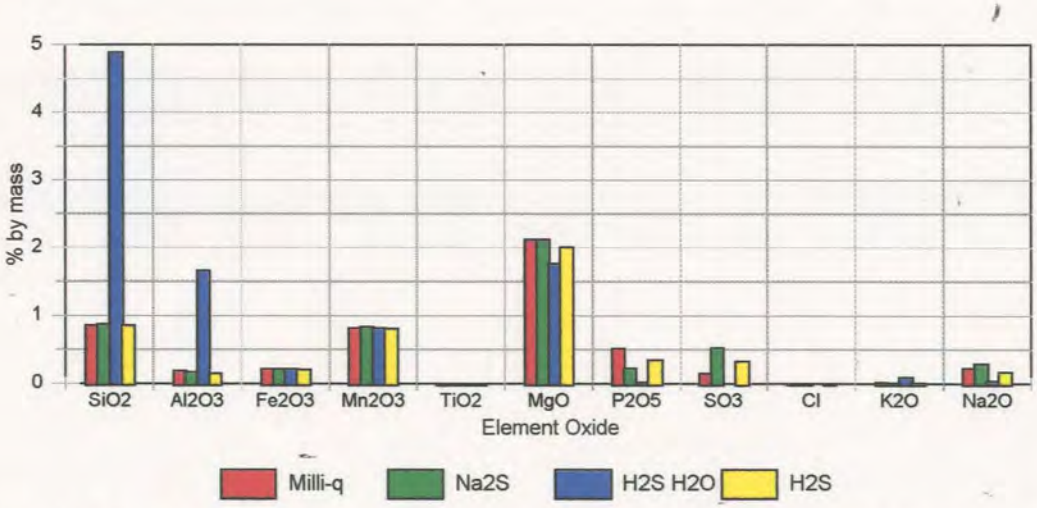

Figure 2.4 XRF analyses of the filter cakes of all sulfide treatment experiments

The filter cake samples contained similar amounts of Mn_2O_3 and Fe_2O_3. Even in the control sample where no sulfide was added to the solution, the majority of the iron and manganese remained in the insoluble portion of the suspension. In general, the levels of the other element oxides were also similar with a few exceptions in the analysis of the sample treated with H_2S water. This sample was too small for conventional bead analysis and it was therefore analysed using a microbead preparation method and this may explain the higher levels of silica and alumina found in this sample. One notable difference between samples exposed to sulfide ions and the sample mixed in ordinary milli-Q water is that the level of SO_3 is higher in the samples exposed to sulfide.

2.3.2.3 Atomic Absorption Analysis of Filter Cakes

Table 2.4 Atomic absorption analysis of filter cake material after sulfide treatment

Solution	% Mn_2O_3	% Fe_2O_3	% CaO
Deionised water	1.516	0.562	69.739
0.2 M Na_2S	1.107	0.528	71.974
H_2S	1.223	0.541	59.094

The atomic absorption analyses confirmed that the iron and manganese content of the samples was similar regardless of whether or not there was sulfide present in the solution. From the observation of black speckles in the filter cakes of the sulfide containing samples, it was assumed that metal sulfides were forming and were remaining in the filter cake. In the sample that was not exposed to sulfide, these metals remained in the oxide form and were also retained in the filter cake.

2.3.2.4 pH Analyses of the Filtrates

Table 2.5 pH analysis of filtrates after sulfide treatment

Solution	pH
Milli-Q water	12.2
0.2 M Na_2S	12.2
H_2S H_2O	12.3
H_2S	12.2

Hydrogen sulfide and water saturated with hydrogen sulfide are both sources of hydrogen ions and could therefore make the medium more acidic by reacting with hydroxide ions. In these experiments however, the concentration of hydroxide ions was so large in comparison to the small amount of H_2S added that there was no noticeable different in the pH of the filtrate.

2.3.2.5 Analysis for Soluble Sulfide in the Filtrates

Table 2.6 Sulfide analysis on filtrates after sulfide treatment

Solution	$S^{2-}/mg.l^{-1}$
Milli-Q water	-
0.2 M Na_2S	345.42
H_2S H_2O	0.53
H_2S	0.09

In the experiments using H_2S water and H_2S, almost all of the sulfide available did bind to cations in the sample suspension leaving only a trace in the filtrate. For comparison, the sulfide content of the H_2S water used in the experiment was 12.76 $mg.l^{-1}$ and the H_2S gas used was 1 % H_2S in N_2 and very little of this was found in the filtrate. In the experiment using Na_2S however, there was a large amount of sulfide left in the filtrate.

For comparison again, the 0.2 M Na_2S solution should contain 643.64 $mg.l^{-1}$ S^{2-} which means that approximately half of the available sulfide was consumed. A less concentrated solution could be used.

2.3.2.6 Atomic absorption Analyses of the Filtrates

Table 2.7 Atomic absorption analysis of filtrates after sulfide treatment

Solution	% Mn_2O_3	% Fe_2O_3	% CaO
Milli-Q water	0.002	0.000	1.426
0.2 M Na_2S	0.000	0.000	1.029
H_2S H_2O	0.000	0.000	1.304
H_2S	0.000	0.000	1.244

Effect of Sulfide on Metal Precipitation: No iron was detected in any of the filtrates. Traces of manganese were found in the filtrate of the milli-Q water experiment. This implies that the form in which manganese occurs in the natural slaked lime sample is slightly soluble. Hydroxide precipitation is not sufficient to keep all traces of the manganese in the solid form. When sulfide in any form is added to the suspension, all residual manganese is rendered insoluble. This is a predictable result as manganese hydroxide is more soluble that manganese sulfide (Callander and Barford, 1983). In terms of the calcium content of the filtrate, it would be expected that the concentration of calcium in the solution would increase in the presence of hydrogen sulfide. The available hydrogen ions from this weak acid should neutralise some of the hydroxide ions in solution shifting the equilibrium:

$$Ca(OH)_2 \rightleftharpoons Ca^{2+} + 2\ OH^-$$

to the right and causing more calcium to come into solution. This effect is not seen in this experiment. It is most likely that as mentioned before, the concentration of H_2S is simply too small to have any measurable effect on the solubility of the calcium. H_2S is a

weak acid and large quantities of gas would be used to lower the pH of an alkaline sample. During the course of this investigation, the ability of H_2S to lower the pH of a slaked lime suspension was tested and the pH was seen to drop but a large volume of gas was used to bring about a small change in pH. The sample then also contained high concentrations of dissolved sulfide which resulted in high levels of sulfur compounds in the calcium carbonate precipitate.

Effect of Sulfide on Calcium Concentration: In terms of calcium ion concentration, this experiment seems to suggest that the addition of a sulfide source decreases the solubility of calcium. This is contrary to theoretical predictions as in the latter two experiments, the amount of hydrogen sulfide added is too little to have a major effect on the calcium in the system. In the case of the Na_2S addition, the concentration of sulfide is much higher and some CaS could be formed. CaS, however, is soluble while $Ca(OH)_2$ is only sparingly soluble, having a solubility of $1.85g/l^{-1}$ in water at 0°C (Weast and Astle, 1981), so the concentration of calcium in the solution should increase in this case. In interpreting these results, it must be noted that the fluctuation in calcium ion concentration is at least partially due to the fact that the concentration of calcium in these samples is very high. This necessitated the use of large dilutions which increased the error in the result. Practically, in order to minimise the dilution factor, highly concentrated standards were used to prepare the calibration curve. The repeatability of readings at these high concentrations was lower than ideal. These analytical problems were consistently seen throughout the results reported in this chapter and it is for this reason that the XRF analyses were added to the battery of tests. XRF is more suited to the analysis of elements found in large concentrations just as the atomic absorption results were more accurate in determining low concentrations. These results should therefore be studied in combination as they complement each other.

The XRF analyses of the filter cakes show that the concentration of calcium in the filter cake is lower when H_2S is used that when milli-Q water is the solvent, possibly indicating the increased solubility of the calcium ions.

Notes on the Experimental Procedure: In the last experiment, it was seen that complete precipitation of iron and manganese was not feasible using hydroxide precipitation. Trials with sulfide precipitation were the next logical step. The procedure used for hydroxide precipitation testing was modified before being applied. One reason for this was the fact that H_2S has extremely low solubility in acidic solutions. The equilibrium between H_2S (gas) and HS^- (dissolved) is governed by the equation (Hilton and Oleszkiewicz, 1988):

$$H_2S = (1+1.02 \times 10^{(pH-7)})^{-1}$$

Already at a pH of 5, almost all sulfide will be present as hydrogen sulfide gas and in a solution with a pH of 0, all of the sulfide would be found as H_2S and would leave the system without being able to react with system components.

A suspension was chosen as the reaction medium because the experiment with the milli-Q water showed that a filtered solution contained very few impurities for removal. This would imply that calcium carbonate prepared using this filtrate would contain have a high level of purity. Contacting the sulfide containing solution with a suspension would give the sulfide more scope to work.

When sulfide ions, in any of the forms tested, were added to the initial solution, metal sulfides formed as black precipitates and there was no iron detected in the filtrate. It was interesting to note that the metal sulfides seemed to accumulate in visible granules. It may be possible to treat a suspension with a source of sulfide ions, form metal sulfide granules and separate these using physical means. This does not fall within the scope of this project but could make a worthwhile investigation.

Presence of Sulfide in the Filtrate: When a sulfide compound was added to the solution, traces of sulfide were detected in the filtrate. These would oxidise spontaneously in the atmosphere (Widdel, 1988) and may precipitate out with the calcium carbonate, decreasing the quality of the product. In a pilot plant scenario, it

would be possible to minimise the amount of sulfide in the precipitate but until this is tested, it would be impossible to say whether or not it could be eliminated from the filtrate completely.

The sulfide could be removed using chemical or biological means but this may not be necessary because it is entirely possible that when the pH of the filtrate is lowered during the carbonation, the sulfide will be lost as hydrogen sulfide gas. In an experiment performed subsequent to this, in which a sulfide containing solution was carbonated, the characteristic odour of hydrogen sulfide was perceived.

2.4 Conclusions

A typical slaked lime sample from Lime Acres contained iron and manganese impurities and these could be solubilised by acid dissolution. Precipitation of these impurities through the use of hydroxide ions did not follow theoretical predictions for a single metal system. Furthermore, hydroxide precipitation resulted in a compromise between the amount of metal removed and the amount of calcium left in solution and was, therefore, not a recommended solution for this system. When the filtrate of a slaked lime suspension was analysed by atomic absorption spectroscopy, no iron was detected and only traces of manganese were found. Treatment of such a slaked lime suspension with sulfide ions resulted in the formation of metal sulfide clusters and the filtered solution of such a treatment was free of metal ions. Sulfide treatment did, however, result in residual sulfide remaining in solutions and this issue may need to be addressed.

Chapter 3: Starting Materials and Effect of Recycling

3.1 Introduction

In the previous chapter, it was seen that if calcium hydroxide is dispersed in water, the undissolved material is removed by filtration and the filtrate is carbonated, the resulting calcium carbonate has a high degree of purity. This method of white lime production is simple and effective and is the basis of many methods (Shimosato and Aiura, 1952; Miki *et al.*, 1972; Gospodaru *et al.*, 1981; Bunger *et al.*, 1997). If a similar procedure is to be used at Lime Acres, the viability of the method using the specific starting materials with their associated impurities, must be established.

There are also disadvantages associated with this procedure which should be minimised if possible. The first of these disadvantages is the cost of the starting material. Lime is produced from the calcination of limestone. The calcination process has associated costs such as the cost of coal (or another energy source), maintainance of the kiln and labour. While the cost per ton of material is not large, it does nonetheless diminish the profit and use of a cheaper source of calcium ions would be beneficial. Lime (slaked or unslaked) is not the only source of calcined limestone available at a lime factory.

Four different sources of calcium ion were available in this study, namely: ground unslaked lime (CaO), slaked lime ($Ca(OH)_2$) and the process stream wastes: precipitator dust from Lime Kiln 9 (LK9 p.d.) and cyclone dust from Lime Kiln 6 (LK6 c.d.). These have been described in Chapter 1. Calcium carbonate ($CaCO_3$) was not investigated as a source as the dissolution of $CaCO_3$ in water gives calcium bicarbonate which is not removed by carbonation. Each material contains different levels of contaminants and is available at a different cost. As waste materials, the latter two source materials were attractive but it is necessary to determine the effect of using the cheaper starting materials on product quality and quantity.

The second disadvantage of the proposed method, is that large volumes of water and large amounts of the calcium hydroxide source are used to produce small amounts of product. If the water and calcium source are discarded after a single use, this would result in a great deal of waste and an inefficient process. One way of combating this would be to reuse the materials. Essentially this would mean establishing a continuous process in which water is saturated with calcium ions from a source, those ions are then removed (as the carbonate) and the water is returned to the same source so that more ions may be dissolved. This would continue until the source is so depleted of available calcium that the process is no longer viable.

There are two major concerns associated with this technique. Firstly, the samples are being recycled and at each stage, more of the desired substance is removed. There is a danger that as the relative content of impurities in the starting material increases, so some of these impurities will be found in the final product. The quality of the final product would then decrease.

The converse of this would be that if there are any contaminants that tend to co-purify with the calcium carbonate, the concentration of these in the starting material would decrease progressively. Successive crops of the final product would thus increase in purity.

Secondly, as the original starting material is recycled, the concentration of available calcium decreases. It is important to know how quickly the calcium will be depleted and if this will have a great effect on the yield of product.

Three studies were performed to investigate the concerns stated above. The first study investigated the purity of calcium carbonate prepared using different starting materials, the aim being to ascertain the degree to which the purity of the starting material affects the purity of the final product. The second study investigated the changes in the quality of the product when the starting material was recycled and the third study investigated the changes in quantity with recycling.

3.2 Source Materials

An investigation to determine what impurities are found in simply prepared calcium carbonate and the effect of different starting materials on these chemical impurities.

3.2.1 Materials and Methods

The solubility of $Ca(OH)_2$ in water is affected by the concentration of Ca^{2+} already found in the solution. Water available at Lime Acres is pumped from an abandoned limestone quarry and therefore contains substantial dissolved $CaCO_3$. It is therefore important to use water with a similar composition in this experiment. For this purpose, 25 l of tap water was poured into a clean plastic container and crushed limestone (6-10 mm fraction) was added to cover the bottom. This was shaken and left for 4 days to equilibrate before beginning further tests. This water will be referred to as "quarry water".

Potential feedstock materials to be evaluated were weighed in 500 g amounts into a plastic bucket and 5 l of "quarry" water was added. The materials used were CaO, $Ca(OH)_2$, cyclone dust, precipitator dust and a 50:50 mixture of the precipitator and cyclone dust samples. The suspension was stirred, using a mechanical stirrer, for 30 min after which the suspension was filtered under vaccuum through a Whatman 42 filter paper. The solid portion was returned to the bucket. The filtrate was transferred to a 5 l glass beaker and CO_2 gas was bubbled through it until the pH dropped to 8.2. The precipitated $CaCO_3$ was collected by vacuum filtration, dried at 60°C and weighed.

3.2.1.1 Sampling

Samples of the starting materials and calcium carbonate product were analysed.

3.2.1.2 Chemical Analysis

Pressed beads were made of the starting materials (method in Appendix A) and elemental analysis was performed using a Siemens MRS 400 MP X-ray Fluorescence Spectrometer (XRF) for elemental analysis. The general program: pressed bead 2, was used.

Pressed pills were made of the calcium carbonate samples and these were also presented to the XRF. These were analysed using a specific program calibrated for natural limestones.

3.2.1 Results and Discussion

3.2.1.1 Starting Materials

The elemental analysis of the starting materials is given in Figure 3.1. The calcium peak was not shown as this peak dominated the composition to such an extent that the other peaks could not be seen clearly.

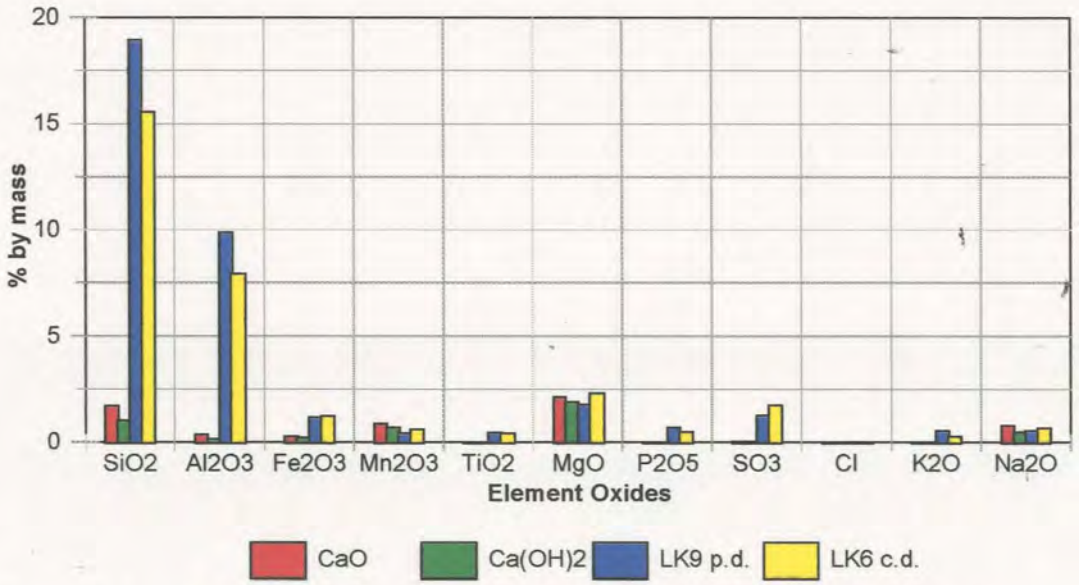

Figure 3.1 Comparison between levels of contaminants in different starting materials

The starting materials had different chemical compositions and different levels of impurities. The lime samples had relatively low levels of impurities as would be predicted for

commercial products. The dust samples on the other hand, had substantially higher levels of silica and alumina as well as higher concentrations of iron oxide. In the kiln, alkali element salts volatilise in the hotter portions of the kiln and are carried in the air stream to the cooler portions where they condense on the small particles in the air stream. These then enter the cyclones and/or precipitators. Alkali metals are, therefore, found in higher concentration in the dust than in the lime products. Sulfate, likewise, originates mainly from the coal ash which forms part of the kiln dust.

3.2.1.2 Calcium Carbonate Product

The elemental analysis of the calcium carbonate is given in Figure 3.2. Again, the calcium peak is not shown so that the other peaks can be seen with greater clarity.

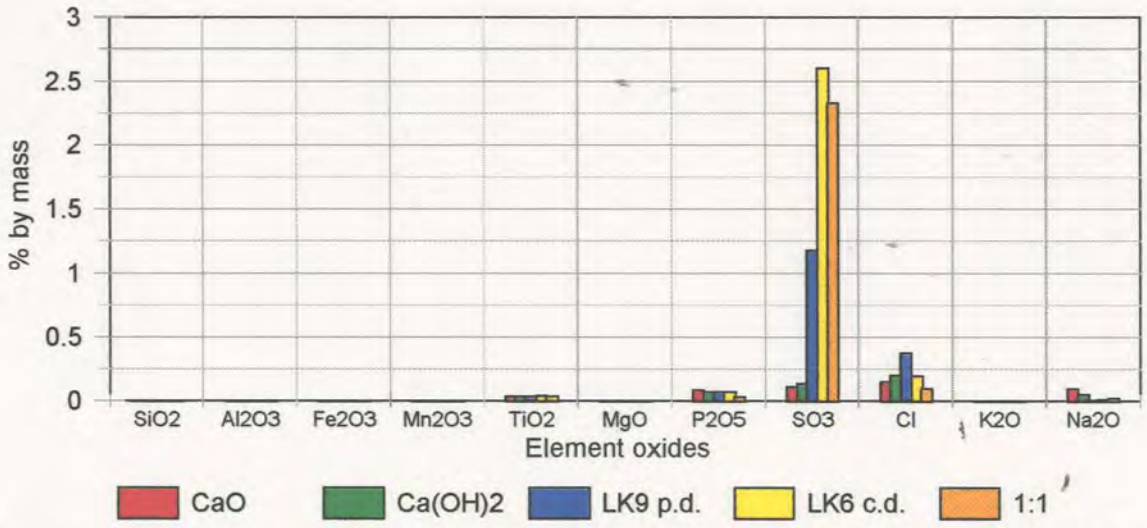

Figure 3.2 Comparison between levels of contaminants in calcium carbonate produced from different starting materials

Notes on the Experimental Procedure: The XRF analysis program used here was calibrated to analyse a natural limestone. The calibration standards were therefore chosen to cover the range usually found in a natural limestone. In this case, the calcium carbonate was far purer than a natural limestone and concentrations of certain elements may have been found in the extrapolated portion of the calibration curve. For this reason, it was not assumed

that the numerical results were absolutely accurate but as all samples were analysed using the same program, comparisons were meaningful.

Impurities: The calcium carbonate samples had similar compositions regardless of the starting material used. Impurities such as SiO_2, Al_2O_3, Mn_2O_3 and Fe_2O_3 are practically insoluble and were removed in the filtration step. The fact that these were found in higher concentrations in certain starting materials was not translated to the product. MgO and K_2O were also not seen in the final product and the levels of TiO_2, P_2O_5 and K_2O were negligable. Cl^- was not seen in the starting materials but was found in the product. There were two reasons for this. Firstly, chloride is volatile at the high temperatures used to prepare the pressed glass beads and chloride initially present in the starting materials could therefore have been lost during sample preparation. Secondly, some chloride could have been introduced into the solution from the Ag/AgCl electrode during the pH monitored step. The only compound found in substantial amounts was SO_3. This SO_3 contamination was specific for the starting materials.

It was encouraging to discover that the quality of calcium carbonate was not greatly affected by the purity of the starting material. The levels of all impurities except sulfur, were low in the product of all experiments conducted. If the sulfate could be removed in some way, dust could be used as a cheap raw material.

Settling Characteristics: Another factor to consider was the settling characteristics of each material studied. Calcium oxide and hydroxide are highly hygroscopic and retain water strongly (Peters and Ku, 1988). This made them difficult and time-consuming to filter. The dust samples contained less available lime and were therefore less hygroscopic and in addition, they contained a variety of components with different sizes and shapes. It was found that the dust samples settled fairly quickly under gravity and most of the liquid was removed easily using the vacuum apparatus whereas the lime samples settled very slowly and incompletely under gravity and filtered extremely slowly even under vacuum. From an industrial perspective, a sample that separates readily is more favourable in terms of energy consumption and process efficiency.

3.3 Effect of Recycling on Quality

An investigation into the effect of recycling water and starting material on the quality of the final product.

3.3.1 Materials and Methods

Three sources of Ca^{2+} ion were investigated: precipitator dust; cyclone dust and a 1:1 mixture of the two. CaO and $Ca(OH)_2$ were not investigated in this experiment as the levels of impurities in these substrates were already low and the effects of recycling on product quality should, therfore, not be great. These materials were also extremely tedious to filter, as mentioned above, and one sample took over ten hours to filter using a vacuum pump. The same method was followed for each of the above materials

Material (500g) was weighed into a plastic bucket and 5 l of "quarry" water was added. The suspension was stirred, using a mechanical stirrer, for 30 min. The suspension was then filtered under vacuum through a Whatman 42 filter paper and the solid portion was returned to the bucket. The filtrate was transferred to a 5 l glass beaker and CO_2 gas was bubbled through it until the pH dropped to 8.2. The precipitated $CaCO_3$ was collected by vacuum filtration, dried at 60°C and weighed. The filtrate was added to the solid material in the bucket and the procedure was repeated.

3.3.1.1 Sampling

The calcium carbonate precipitated at the end of each cycle was analysed.

3.3.1.2 Chemical Analysis

Pressed pills were made of all samples and were presented to the XRF spectrometer used previously. Analyses were performed using a program calibrated for natural limestone analysis.

3.3.2 Results and Discussion

The XRF program used for the analyses was calibrated to analyse a natural limestone. As in section 3.2, it was not assumed that the numerical results were absolutely accurate but as all samples were analysed using the same program, the results were comparable. Complete elemental analyses were carried out and the results that were greater than zero (titanium, phosphorus, chloride and sodium) were compared between the successive cycles. The levels of calcium remained roughly constant throughout and this was not included in the graphs.

3.3.2.1 Precipitator Dust

The analysis of the levels of four elements is given in figure 3.3.

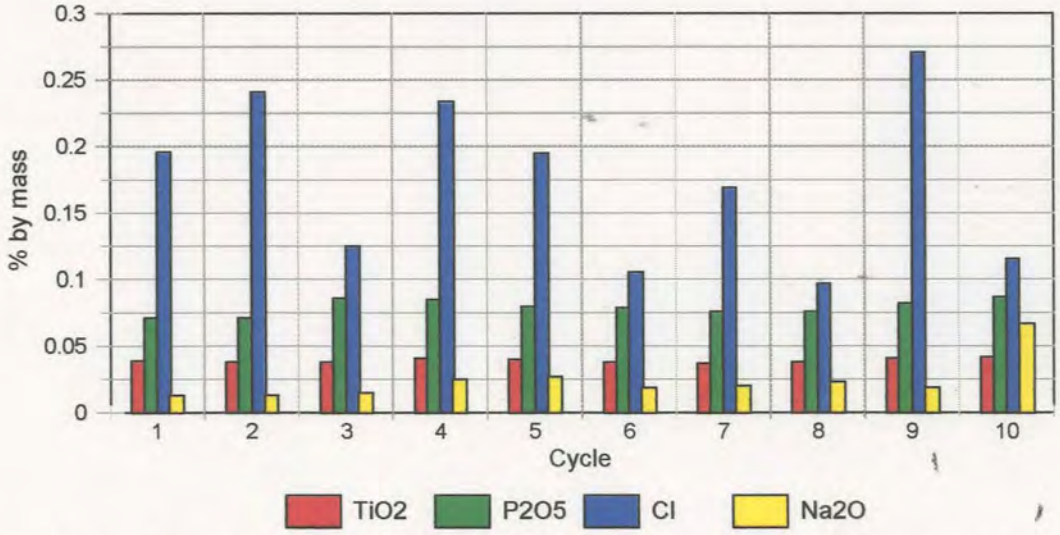

Figure 3.3 Variation in levels of minor element oxides in calcium carbonate produced from precipitator dust

Fluctuations were small and random and there was no apparent relationship between the levels of impurities and the crop number.

The levels of SO_3 are shown in Figure 3.4

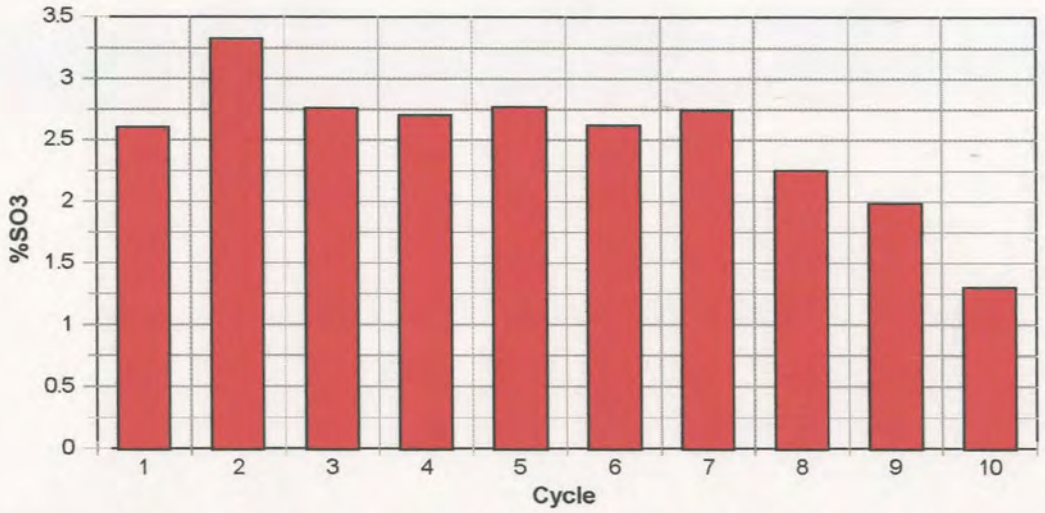

Figure 3.4 Variation in levels of SO_3 in calcium carbonate produced from precipitator dust

The level of SO_3 in the samples decreased with crop number.

Overall, it seemed that the purity of the product increased as the materials were recycled.

3.3.2.2 Cyclone Dust

The variation in levels of minor elements is shown in Figure 3.5.

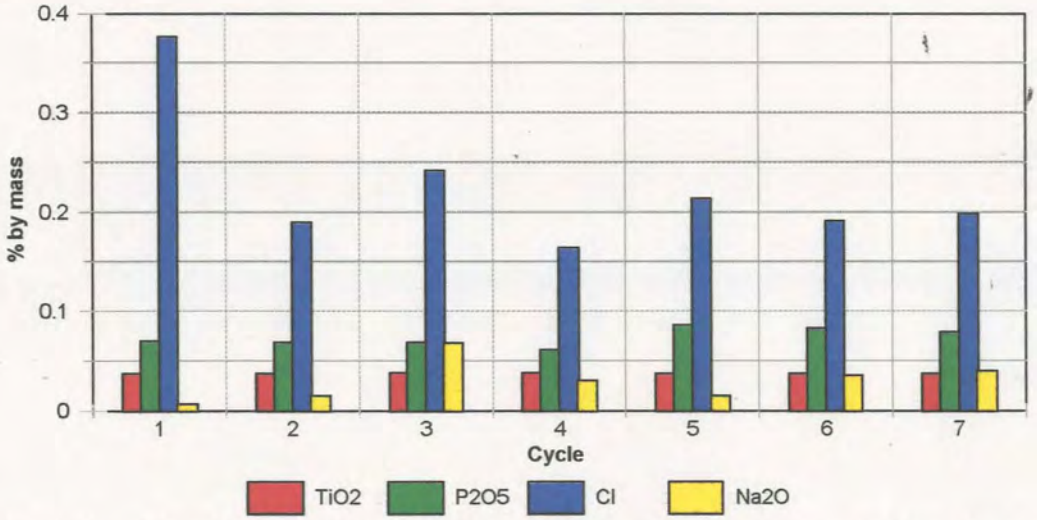

Figure 3.5 Variation in levels of minor element oxides in calcium carbonate produced from cyclone dust

Again, fluctuations were small and random and there was no apparent relationship between the levels of impurities and the crop number.

The levels of SO_3 are shown in Figure 3.6

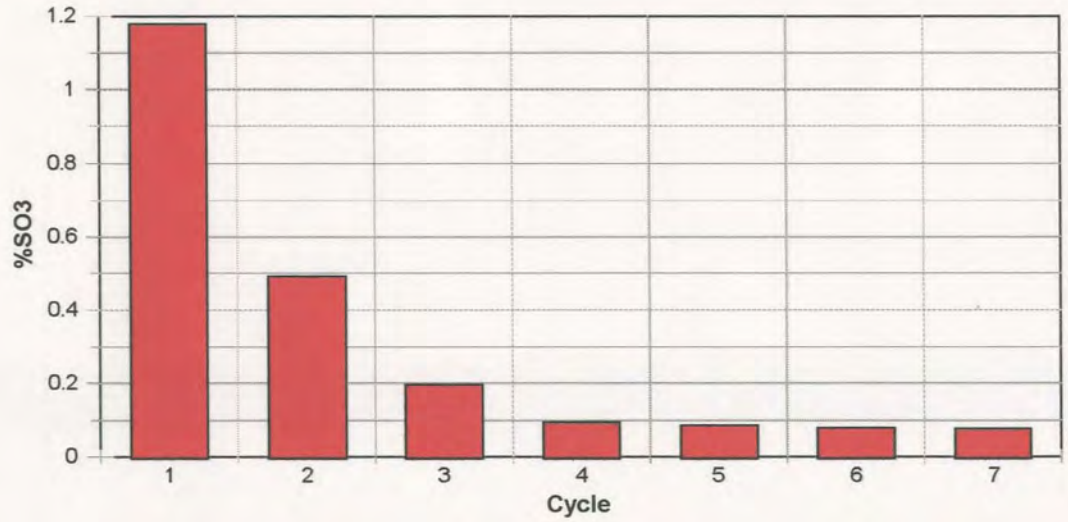

Figure 3.6 Variation in levels of SO_3 in calcium carbonate produced from cyclone dust

The level of SO_3 in the samples decreased with crop number and the initial change was rapid.

Overall, it seemed the purity of the product increased as the materials were recycled.

3.3.2.3 Mixture (1:1) of Precipitator Dust and Cyclone Dust

The variation in levels of minor elements is shown in Figure 3.7.

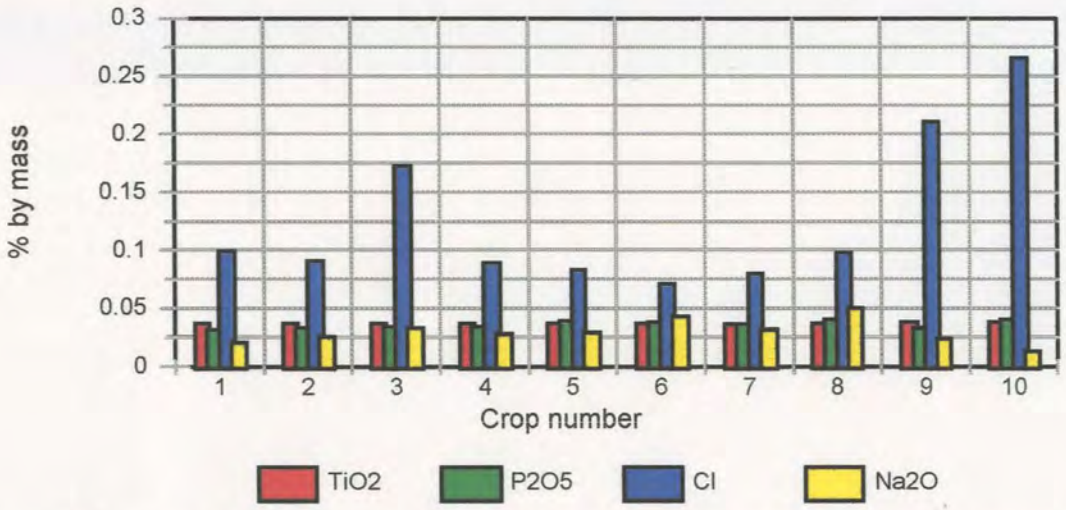

Figure 3.7 Variation in levels of minor element oxides in calcium carbonate produced from a 1:1 mixture of precipitator dust and cyclone dust

As before, fluctuations were small and random and there was no apparent relationship between the levels of impurities and the crop number.

The levels of SO_3 are shown in Figure 3.8

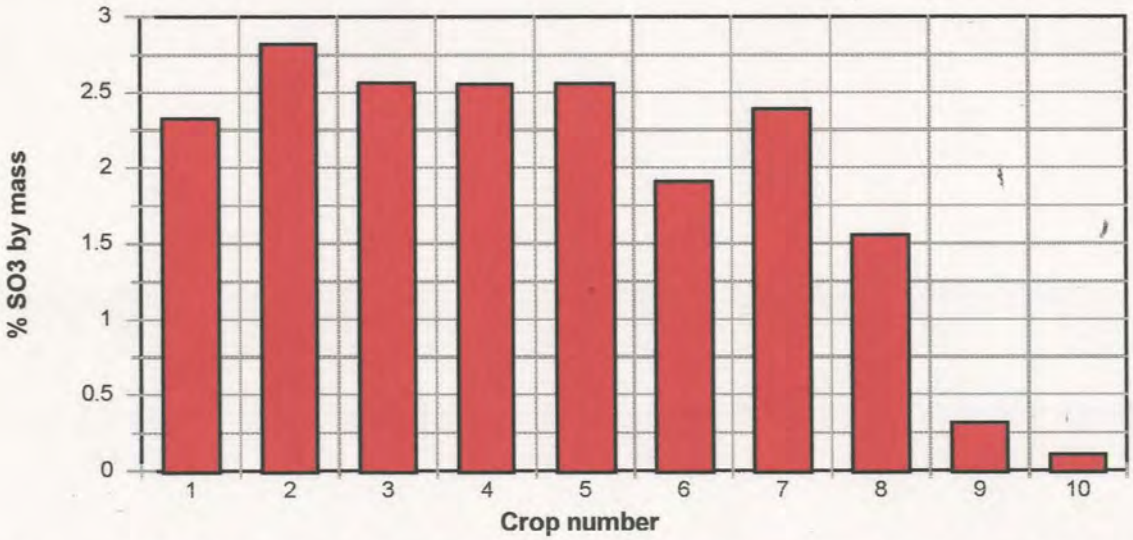

Figure 3.8 Variation in levels of SO_3 in calcium carbonate produced from a 1:1 mixture of precipitator dust and cyclone dust

As before, the level of sulfate in the samples decreased as the cycle number increased. The profile was similar to that of the precipitator dust sample but the final sulfate levels were lower. The settling characteristics and speed of filtration for this mixture were better than for either individual sample. It seemed that the variation in particle size resulting from the combination of two different samples was advantageous to the settling speed.

3.4 Effect of Recycling on Quantity

An investigation into the effect of recycling water and starting material on the quantity of the final product.

3.4.1 Materials and Methods

The method described in section 3.3.1 was used and the final product was dried and weighed.

3.4.1.1 Sampling

Samples of the raw materials were taken for analysis and the calcium carbonate precipitated at the end of each cycle was analysed.

3.4.1.2 Chemical analysis

The calcium carbonate samples were weighed. The free lime in the raw materials was analysed using a Scientec RSA FLT 300 free lime analyser as was the remaining free lime in the material left after the cycling experiments.

3.4.2 Results and Discussion

3.4.2.1 Yield of Calcium Carbonate

The yields when precipitator dust was used as the starting material are shown in table 3.1 and figure 3.9.

Table 3.1 Yields of Calcium Carbonate obtained when Precipitator Dust was recycled

Cycle	1	2	3	4	5
Yield/g	12.3421	10.6395	9.8149	9.1912	7.9124
Crop	6	7	8	9	10
Yield/g	8.2199	7.8066	7.4936	7.2398	4.8248
Sum/g					85.4848

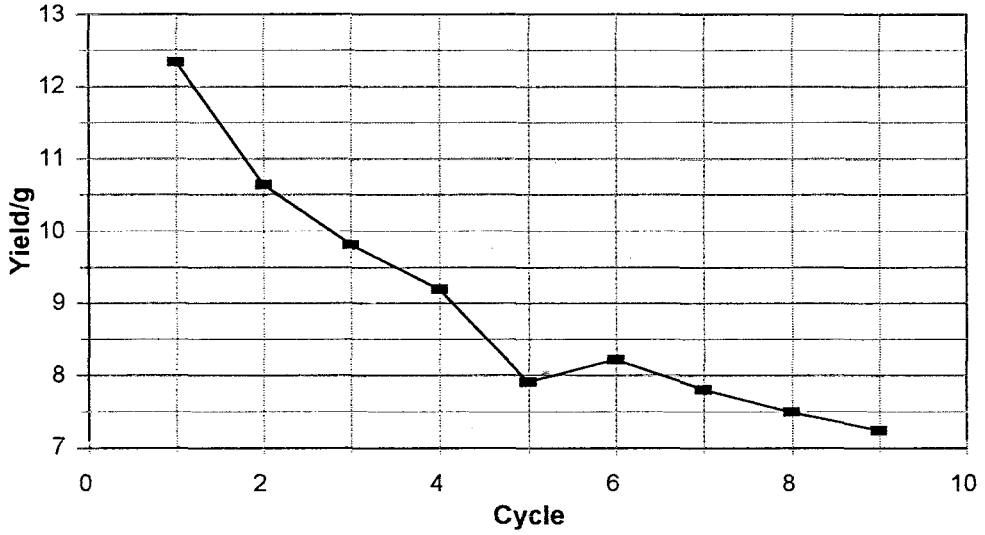

Figure 3.9 Change in yield of calcium carbonate using precipitator dust as starting material

The yield of product decreased with increasing cycle number. There were two factors that contributed to this. Firstly, from a practical side, water was lost through evaporation and spillage and the amount of water available to dissolve Ca^{2+} ions which were precipitated in crop 1 was more than that available for crop 2. Practically, water losses were small and this could be remedied by adding a little water to the system when necessary. The second and major reason was that as the available lime in the solution decreased, so the equilibrium between solid $Ca(OH)_2$ and dissolved $Ca^{2+} + 2OH^-$ shifted and less Ca^{2+} was solubilised, leaving less available for carbonation.

The yield of cycle 5 shows the importance of pH control. The pH was allowed to drop to slightly below 8.2 and bicarbonate ions started forming immediately. Other than this point,

the yield decreased in a predictable fashion until the 10th cycle and at this point, a large decrease in yield was noted. This may be the point at which recycling becomes non-viable.

The yields when cyclone dust was the starting material are shown in table 3.2 and figure 3.10.

Table 3.2 Yields of Calcium Carbonate obtained when Cyclone Dust was recycled

Cycle	1	2	3	4	5	6	7
Yield/g	12.6677	9.3280	8.7032	8.5235	8.1582	7.3780	7.1800
Sum/g							61.9386

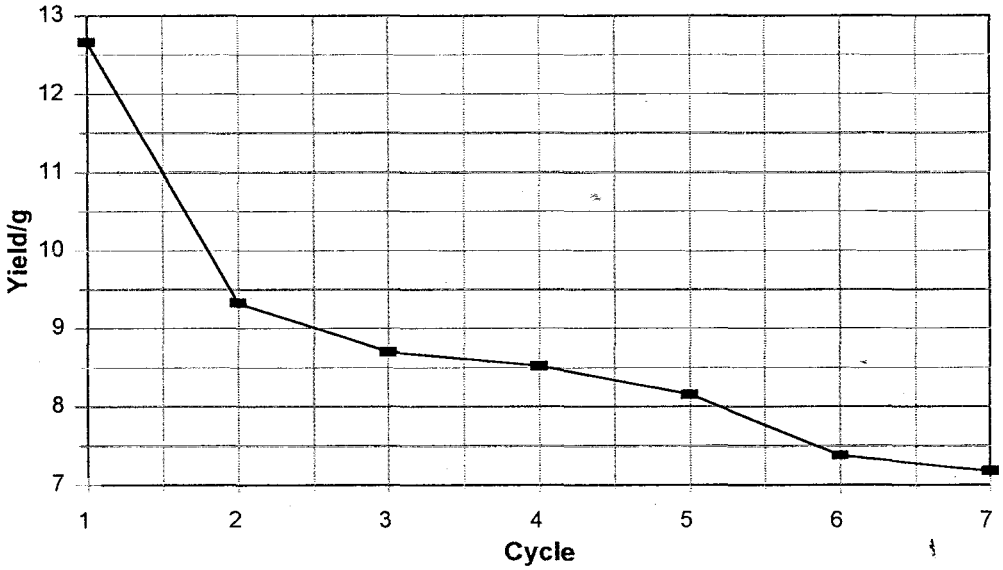

Figure 3.10 Change in yield of calcium carbonate using cyclone dust as starting material

As before, the yield of calcium carbonate decreased with cycle number. The decrease was sharper with this raw material but the initial yield was higher. No obvious drop in yield was seen within 7 cycles. The free lime of the material was still fairly high at this point and the cycles could be continued to increase the overall yield.

The yields when a 1:1 mixture of the two dust samples was used as the starting material, are shown in table 3.3 and figure 3.11.

Table 3.3 Yields of Calcium Carbonate obtained when a 1:1 mixture of precipitator dust and cyclone dust was recycled

Cycle	1	2	3	4	5
Yield/g	12.685	10.2814	10.2073	9.2558	9.1768
Cycle	6	7	8	9	10
Yield/g	8.5939	8.4037	7.8092	7.4563	7.2518
Sum/g					91.1212 g

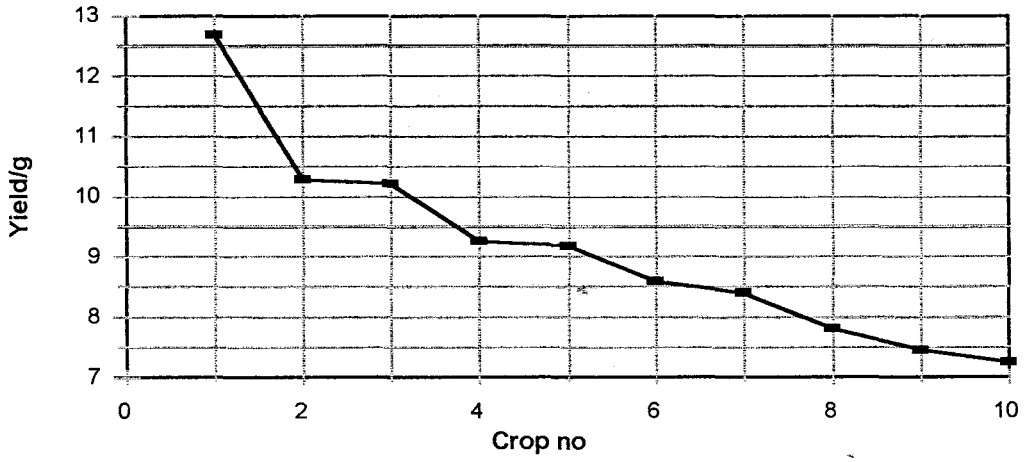

Figure 3.11 Change in yield of calcium carbonate using the 1:1 dust mixture as starting material

The yield decreased as the cycle number increased but the best qualities of each of the dust samples was seen in this combined sample. The initial yield was high in keeping with the cyclone dust sample however, the decline in yield was not as sharp and even the seventh cycle had a yield of over 8 grams of calcium carbonate.

3.4.2.2 Free Lime

The free lime in the starting materials and the final materials (after 10 cycles in the case of precipitator dust and 7 cycles in the case of cyclone dust) is shown in Tables 3.4 and 3.5. In addition, calculations of the theoretical yield of $CaCO_3$ based on the initial free lime value are

shown. The "$CaCO_3$ left" was based on the final free lime value and represents that $CaCO_3$ which could be produced if further cycles were performed.

Table 3.4 Free lime and calcium carbonate yields using precipitator dust as starting material

% Free Lime		Yield of $CaCO_3$/g		
Initial	Final	Theoretical	Actual	Left
11.45	1.3	102.16	85.48	11.60

Table 3.5 Free lime and calcium carbonate yields using cyclone dust as starting material

% Free Lime		Yield of $CaCO_3$/g		
Initial	Final	Theoretical	Actual	Left
21.25	7.7	189.60	61.94	68.70

The % free lime should be a good indicator of the point at which recycle is no longer profitable because of the low recoveries. It can be seen that the final free lime in the experiment using precipitator dust, was low. This sample had suddenly decreased its yield of product to under 5 grams. The calculation of the calcium theoretically remaining in the sample was small. In the case of the cyclone dust however, the final % free lime was higher and there was potential for recovery of another 67 grams of product. It follows that free lime measurements could therefore be used in an industrial setting to regulate changing of starting material.

Purity: As expected from the precipitation studies, the heavy metal contaminants in the raw materials did not dissolve in water to a significant extent and the calcium carbonate precipitated from a filtered lime solution did not contain high levels of metal impurities. These metals may occur in concentrations too low for measurement using an X-ray fluorescence spectrometer and it is possible that they may be detected by atomic absorption. If traces were found however, they could be removed by sulfide precipitation.

Calcium carbonate with a high level of purity was produced by dispersing slaked or unslaked lime in water, filtering and carbonating the filtrate. The product required no further treatment other than drying. This process is inefficient in that lime is already a value-added product and, therefore, has an inherent cost. It is sparingly soluble, necessitating the use of large volumes of water which it retains strongly. This raw material also filters slowly even under a vacuum and is, therefore, not ideal for industrial use.

Advantage of Using Dust as the Starting Material: When a waste material is used as the starting raw material, however, the cost of the process decreases. The waste dusts also retained water less strongly and settled quicker under gravity, making them suitable for an industrial application. There is also the added benefit of reducing the amount of waste dust accumulating at Lime Acres. Currently, this dust accumulates on large waste dumps where it is not only an eyesore and waste of space but can create a hazard when blown by the wind. It cannot be used as landfill because of the high free lime content which would make the water table strongly alkaline. If this dust was used to make calcium carbonate the quantity of dust would be decreased somewhat, a valuable product would be produced and the waste from the process would have a much lower free lime content and may then be used as landfill. Recycling of the water in the process gives a closed system and decreases the amount of water used.

Disadvantage of Using Dust as Starting Material: The main disadvantage of using dust as the raw material in calcium carbonate manufacture is the fact that sulfate is found in the precipitate. Levels can become quite high (2.5 % in one experiment) and this may need to be removed. As was seen in the experiment, one way of minimising the sulfate content was to recycle the raw materials. With each successive cycle, the amount of sulfate found in the raw material decreased resulting in less being available to dissolve in the solution and precipitate in the product. Of course, as sulfate levels decreased, so did the yield of calcium carbonate and this may not be the most economical option.

Sulfate removal may prove to be feasible. There are chemical methods of achieving this but it is not within the scope of this work to investigate these. What is of interest to us is the possibility of using sulfate reducing bacteria to remove the sulfate from the calcium

carbonate. This has not been attempted previously. An added benefit of employing these bacteria is the fact that sulfide ions produced from the reduction of sulfate would precipitate any residual metals in the solution and would give an even purer product.

3.5 Conclusions

Production of calcium carbonate that was almost free of metallic impurities was possible using the range of raw materials evaluated and a simple preparation procedure. Lime-containing waste products, which are readily available at no cost, could be used as raw materials in the process. These raw materials and the water used could be recycled without adversely affecting the purity of the product. The free lime content of the raw materials could be measured at intervals and this could be used to indicate when the calcium content of the starting materials has been depleted. Sulfate was the only major impurity when waste dust was used as the raw material in the procedure used.

Chapter 4: Biological Studies

4.1 Introduction

It has been shown in Chapters 2 and 3 that sulfide precipitation is an effective means of removing iron and manganese from lime solutions. In addition, it was shown that sulfate was the main impurity in calcium carbonate produced by the carbonation of solutions of lime-containing products available at Lime Acres. These findings together indicate a need for removal of sulfate and precipitation of metals to obtain a purer product. A simple method of achieving both of these aims is to use sulfate reducing bacteria (SRB) to reduce the sulfate in the calcium carbonate to sulfide which will then be available to react with any metals in solution, removing them as insoluble precipitates. The ability of SRB to function in this capacity was ascertained in five experiments.

First, a viable culture of SRB was obtained. Sewage was chosen as the source of the SRB used. SRB are found as one of the anaerobic populations in sewage as is evidenced by the distinctive "rotten-egg" smell which is contributed by the hydrogen sulfide produced by the reduction of sulfate. The SRB here utilise the low molecular weight compounds produced by fermentative bacteria (Sterrott and Lester, 1988). As SRB are not the only bacteria found in sewage and many other species compete for available nutrients, it is useful to increase the proportion of SRB at the expense of the other populations. This is achieved by making the growth medium increasingly unfavourable for other populations allowing the SRB to dominate the culture. In this case, the main competitors of the SRB are methanogenic bacteria and the growth of these is inhibited by a high concentration of sulfate (Harada, 1994).

Preliminary chemical tests have shown that levels of sulfate in the precipitate are often around 2 %. A second experiment was therefore performed to determine the ability of SRB to grow in solutions with this sulfate level. The aim was that even if SRB were not

naturally able to grow at elevated sulfate levels, some members of the population would be able to grow and these would multiply and dominate the culture.

The bacterial culture at this point was adapted to heterotrophic growth. In order to minimise the running costs of a future plant using SRB, autotrophic growth was investigated in the third experiment. If the culture could grow using producer gas as its nutrient source, the outlay would only be the cost of a producer gas plant and then coal for subsequent producer gas manufacture. Coal is available at Lime Acres.

SRB have been seen to reduce sulfate without the presence of an organic carbon source (van Houten, 1996). The result is an essentially clean process which can then be integrated into the production of pure (sulfate free) calcium carbonate. There will be no need to add nutrients to enable the SRB to grow and the potential for impurities entering the system is then minimised. Autotrophic SRB containing cultures usually use CO_2 and H_2 as nutrients. In this case, CO_2 addition is not advisable as it would cause the pH to rise and the $CaCO_3$ would redissolve. The lack of this nutrient should not be a problem for the SRB, however, as the $CaCO_3$ suspension will be saturated in HCO_3^- and this should provide an ample carbon source.

The final two experiments cover integration of SRB into a process train for the production of SRB. The possibilities of growing SRB in solutions with elevated pH and in calcium carbonate suspensions were investigated.

4.2 Enrichment of Sulfate Reducing Bacteria

Enrichment for sulfate reducing bacteria (SRB) from sewage

4.2.1 Materials and Methods

A 2 l anaerobic culture vessel was constructed for the growth of the culture (See figure 4.1). 1.8 l of SRB medium, consisting of tap water containing 0.2 % dissolved sulfate

(added in the form of anhydrous sodium sulfate), was placed in the flask and was inoculated with 200 ml sewage sludge. The flask was incubated at 22°C and stirred on a magnetic stirrer. The growth of the SRB in the culture was monitored daily by performing cell counts, measuring pH, determining sulfide and sulfate concentrations and measuring the chemical oxygen demand (COD). These methods are described in Appendix A.

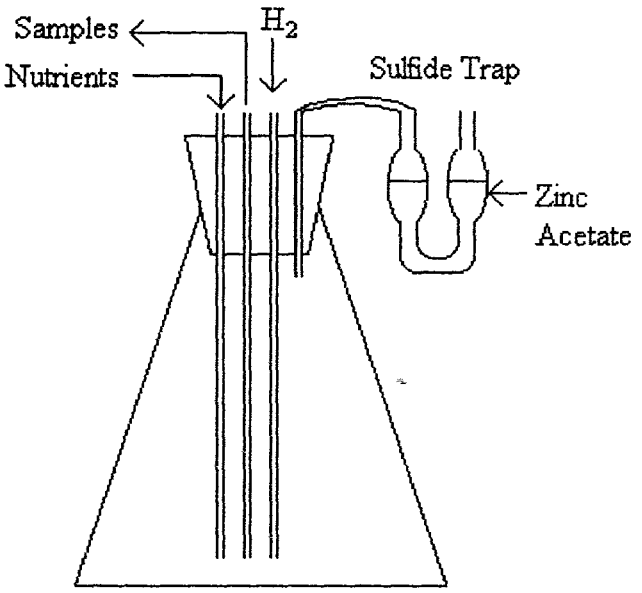

Figure 4.1 Anaerobic flask used in the SRB experiments

4.2.2 Results and Discussion

The results of the cell counts, pH, S^{2-}, SO_4^{2+} and COD measurements are depicted in figures 4.2 to 4.6.

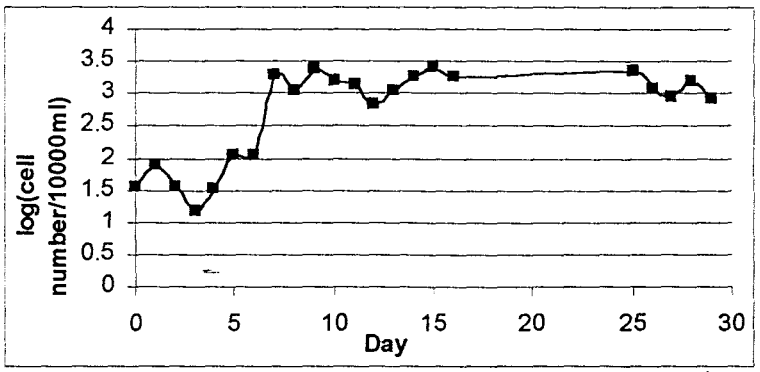

Figure 4.2 Cell Counts during SRB enrichment

The cells immediately began growing and multiplying but this increase soon stopped and the cell numbers began to decline (figure 4.2). By the fifth day, the culture was still in a lag phase and it was thought that the bacteria may have depleted the nutrients originally supplied in the sewage. This suspicion was supported by the observation that although the liquid in the flask was initially grey and turbid with suspended matter, by the second day, it had become clear. Chemical oxidation demand (COD) measurements were therefore added to the battery of tests performed on the culture and it was found that the nutrients had indeed been depleted. After a survey of the literature, it was discovered that yeast extract was often used as a nutrient source for SRB (Mechalas and Rittenberg, 1960; Postgate, 1963; Herrera *et al.*, 1991; Panchanadikar and Kar, 1993) and the most common addition level was 1g per litre of culture medium. Yeast extract (20 g) was therefore dissolved in a small volume of water and added on day six. The cell numbers immediately began to increase. The culture again entered a lag phase and cell numbers remained stable even after a further nutrient addition on the fifteenth day.

The initial culture was a mixed one, consisting of many bacterial types. As the enrichment progressed, so the sulfide concentration increased to levels that were toxic to other cells and the source of organic carbon was changed to one that was favourable for SRB growth. The trustworthiness of cell counts is questionable as "direct microscopic identification of SRB in samples is almost impossible" (Widdel, 1988). The cells counted were the vibrios and rod shaped cells but these cell forms are not specific for SRB. In fact, the greatest increase in cell numbers in this study, was observed at a time when sulfide concentration was low (Figure 4.4). It is unlikely that this increase in cell number was only representative of the number of SRB. As the enrichment proceeded, it was noticed that number of short rods was decreasing and the number of longer rods was increasing. In retrospect, the short rods were probably not SRB. This was not known at the time as there are species of SRB that are found as short, oval rods (Widdel, 1988). As a method of determining SRB proliferation, this method is not recommended, however, if the majority of cells in the culture are SRB, this method does allow monitoring of their growth or decline and can be used in this context.

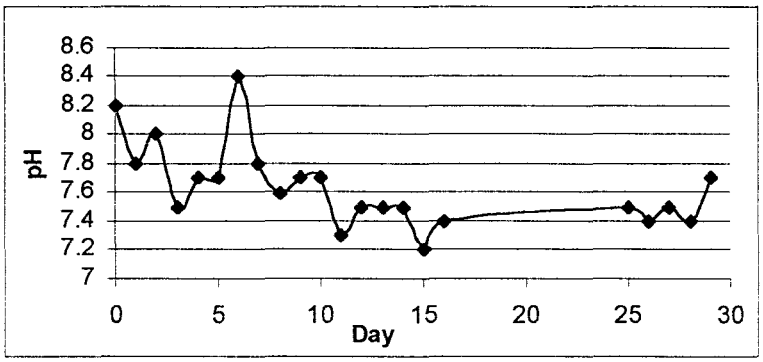

Figure 4.3 pH during SRB enrichment

Sulfate reduction should cause the pH of the medium to increase as the reduction of sulfate results in the formation of sulfide ions. While a portion of these ions will dissolve in the liquid phase, the remainder will escape as hydrogen sulfide gas. Each mole of hydrogen sulfide that leaves the liquid phase translates into two moles of hydrogen ions that are removed from the system, corresponding to an increase in pH. It must be remembered, however that the amount of hydrogen sulfide able to leave as a gas is governed by the equilibrium (Hilton and Oleszkiewicz, 1988):

$$H_2S = (1 + 1.02 \times 10^{(pH-7)})^{-1}$$

The initial pH of the medium was 8.2 and at this pH, 5.8 % of the sulfide would leave as H_2S. This meant that the removal of hydrogen ions from the solution and the corresponding pH elevation would happen slowly. Concurrently, complete oxidation of organic nutrients was being carried out by SRB and other sewage bacteria, causing the production of carbon dioxide (Widdel, 1988). Some of this CO_2 would dissolve in the water to form carbonic acid and the pH would drop:

$$CO_2 + H_2O \rightarrow H_2CO_3 \rightarrow H^+ + HCO_3^-$$

As this decrease occurred however, the sulfide would become less soluble and more would be lost as hydrogen sulfide. With time, the pH decrease would become progressively slower, would pass through a stable area where the pH does not change

much and would then begin to rise (figure 4.3). This is precisely what was seen in the flask and by the twenty-ninth day, the pH was beginning to rise.

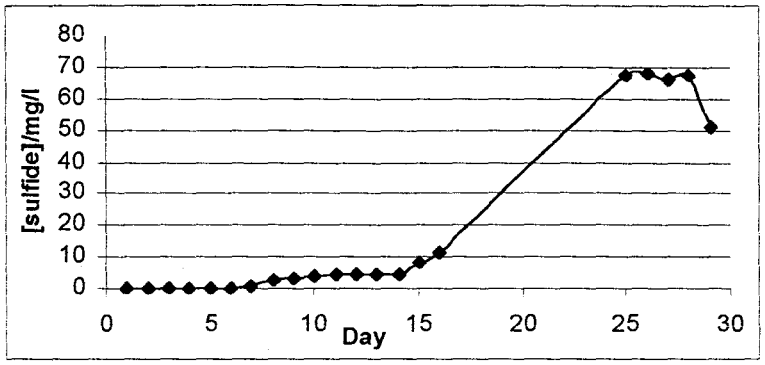

Figure 4.4 Sulfide concentration during SRB enrichment

SRB are known to be prone to lags or periods of adaptation when transferred to new growth conditions (Brandis and Thauer, 1981). It is therefore not unexpected that little sulfide production is seen in the first two weeks of growth. During this time, the culture was still adapting to the changed culture conditions. No effort was made to make the culture anaerobic from the start as heterotrophs would utilise the oxygen in the medium giving rise to an anaerobic culture in which SRB would begin to flourish (Wakao et al, 1979). It is therefore likely that the SRB grew and reduced sulfate slowly at first and then as culture conditions became more favourable, they gained the competitive advantage and proliferated producing sulfide which was measured in the liquid phase. After day 24, no further increase in sulfide concentration was observed. This does not imply that the culture had ceased to produce sulfide because it must be remembered that by this stage, the pH of the culture had dropped to around 7.4. At this pH, 26 % of all sulfide formed would leave the solution as hydrogen sulfide gas. This was confirmed by the observation that white zinc sulfide formed in the zinc acetate gas trap. For a constant sulfide concentration to be maintained, sulfide must be produced daily, indicating that a stable, active sulfate reducing population had been achieved.

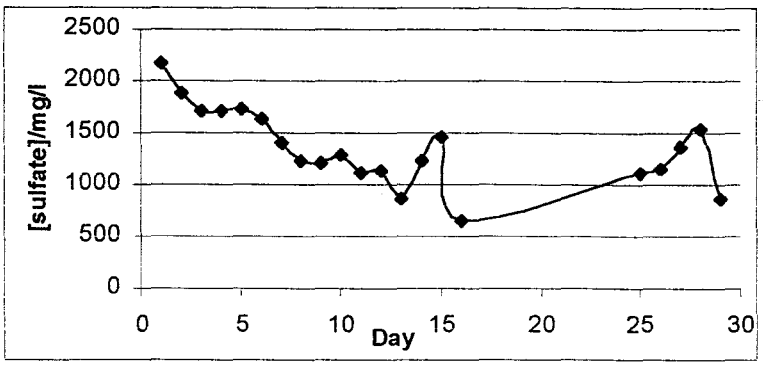

Figure 4.5 Sulfate concentration during SRB enrichment

A sulfate concentration of 2 g per litre was chosen for the enrichment as this sulfate level allows the growth of SRB while methanogens are inhibited (Harada *et al.*, 1994). Sulfate measurements showed that the sulfate concentration in the solution decreased slowly during the course of the enrichment and then began to fluctuate (figure 4.5). The decrease was in keeping with the fact that sulfide was produced and the fluctuation may have been an indication that sulfide oxidising bacteria were present and that these oxidised some of the sulfide back to sulfate. The medium in the flask was seen to have a slight reddish colouration which could indicate the presence of purple sulfide oxidising bacteria (Widdel, 1988) and a buildup of a creamy substance was observed on the surface of the liquid medium. Growth of green and purple sulfur bacteria in such cultures would not be unusual and even low light intensity would support such growth (Du Preez *et al.*, 1992). Even though the flask was covered in tinfoil, it would be possible for sufficient light to penetrate to allow the bacteria to grow.

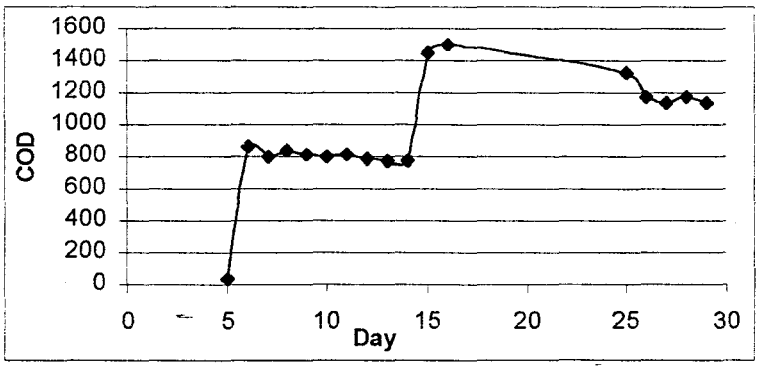

Figure 4.6 Chemical Oxygen Demand during SRB enrichment

Figure 4.6 shows that before the addition of yeast extract on the sixth day, nutrients were limiting in the culture flask. After the nutrient addition, the culture began to grow and the COD gradually dropped. When the growth of the culture entered a lag phase, more yeast extract was added (fifteenth day) as can be seen in the above figure but this did not have a large stimulatory effect on growth and was not repeated.

Once the culture had been growing for a month, it was seen that a stable population of bacteria which actively reduced sulfate to sulfide had been established and further experiments could now be conducted.

4.3 Sulfate Tolerance Studies

Selection for those SRB able to grow in sulfate concentrations up to 2 %

4.3.1 Materials and Methods

Anaerobic culture vessels (2 l volume) were constructed for the growth of the cultures. Three different SRB media were prepared containing 0.2, 1 and 2 % sulfate (added in the form of anhydrous sodium sulfate) dissolved in tap water and in each case, 1.8 l of medium was inoculated with 200 ml sewage sludge. The flasks were incubated at 22°C and stirred using a magnetic stirrer. The growth of the SRB in the cultures was monitored daily by performing cell counts, measuring pH, determining sulfide and sulfate concentrations and measuring the chemical oxygen demand (COD).

4.3.2 Results and Discussion

The results are showed in figures 4.7 to 4.11.

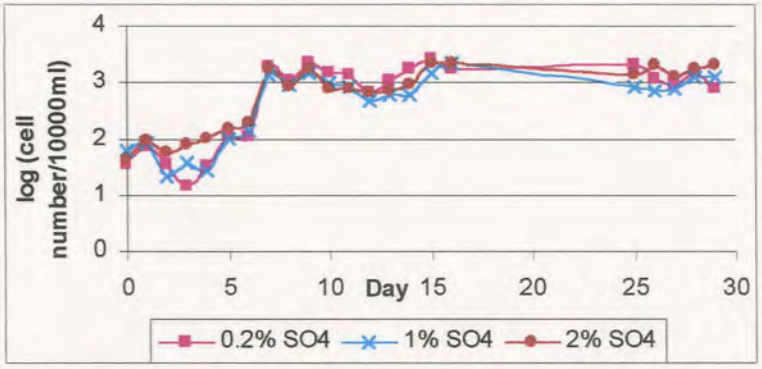

Figure 4.7 Cell Counts of cultures with different sulfate levels

As seen in section 4.2, there was an initial lag time during which grown was slow but the cell numbers did begin to increase after nutrients were added on the sixth day (figure 4.7). Growth was similar in all flasks, regardless of the sulfate concentration. Levels of sulfate up to 2 % were not toxic to the cells.

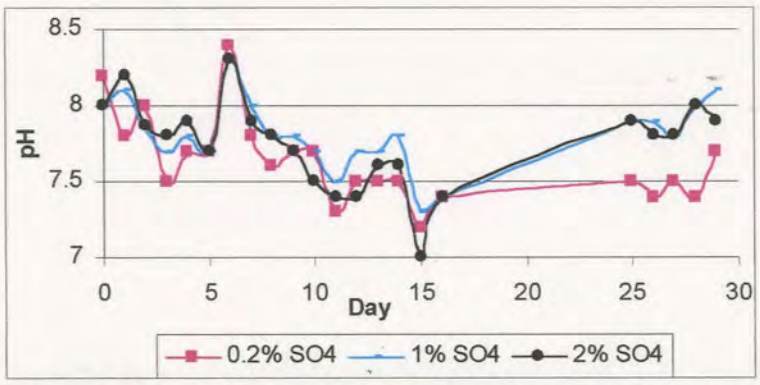

Figure 4.8 pH of cultures with different sulfate levels

As explained in section 4.2, the pH initially decreased and then began to increase as sulfide production became a major reaction (figure 4.8). Alkalinisation of the medium occurred faster in the cultures with a higher sulfate concentration.

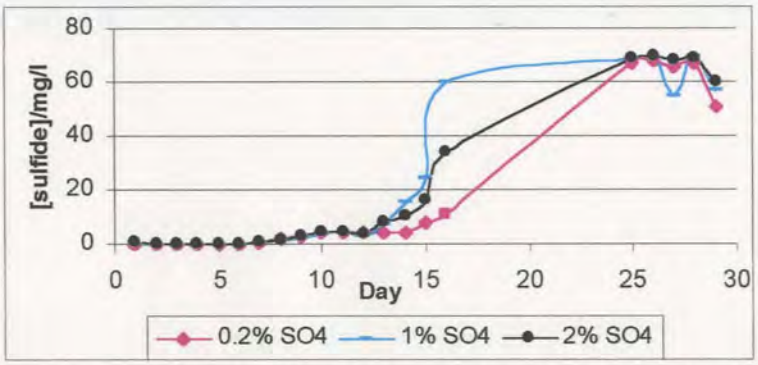

Figure 4.9 Sulfide production in cultures with different sulfate levels

All of the cultures began to produce sulfide after a similar lag time, regardless of the concentration of sulfate (figure 4.9). The sulfate concentration did however affect the rate of sulfide production once this started. As the concentration of sulfate increased, so the initial rate of sulfide production increased. All three flasks eventually stabilised at the same sulfide concentration. Two possible causes of this were toxicity and product inhibition. Sulfide toxicity depends on the concentration of unionised hydrogen sulfide which was approximately 25 % of total sulfide production in this case. Conversely, product inhibition depends on the concentration of dissolved sulfide in the medium (Hilton and Oleskiewicz, 1988), which was 70 mg/l in this case. Both probably contributed to the stabilisation.

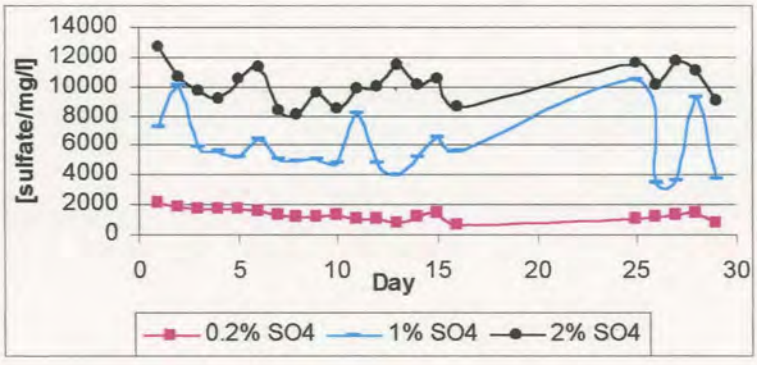

Figure 4.10 Sulfate concentration in cultures with different initial sulfate levels

The sulfate concentration was found to decrease with time (figure 4.10). There was a great deal of instability in the sulfate curve. This was partially due to the presence of

green and purple sulfur bacteria that re-oxidised some of the sulfide produced to sulfate. As mentioned previously, the flask containing 0.2 % sulfate acquired a reddish tinge and it was also noticed that the flask containing 1 % sulfate became greenish with time. The action of sulfur bacteria was not enough to account for all of the fluctuation, however, as results were sometimes higher than the initial concentration of sulfate added. These fluctuations were attributed to the method of analysis employed. The turbidometric determination of sulfate did not seem to be well suited to multi-component systems. It is accurate when simple aqueous sulfate solutions are tested but when solutions containing many other components are tested, these other chemical constituents may co-precipitate with the barium sulfate and give falsely high results. Sulfate reduction as analysed using this method is not recommended as a determination of growth of sulfate reducing bacteria.

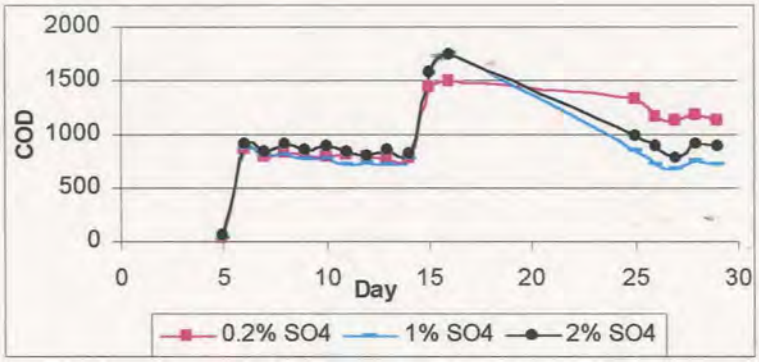

Figure 4.11 Chemical Oxygen Demand of cultures with different sulfate levels

The two cultures with the higher sulfate concentrations utilised the available nutrients more rapidly than the flask with the lowest concentration (figure 4.11). This is not surprising as the rate of sulfide production was most rapid in these flasks indicating actively growing cultures. Increased sulfate concentrations had a favourable effect on the growth of the SRB. It has been reported that methanogenisis is inhibited at SO_4^{2-}/COD ratios above 0.1 (Harada *et al.*, 1994). Even after the second nutrient addition in the flask containing the lowest sulfate concentration, this ratio is still over ten fold higher. SRB should therefore have the competitive advantage in all flasks and this advantage increases as the sulfate concentration in increased, resulting in better growth.

4.4 Growth of SRB under autotrophic conditions

An investigation of the possibility of growing SRB without the addition of yeast extract, simply with H_2 and inorganic dissolved carbonate.

4.4.1 Materials and Methods

H_2 was bubbled through the culture flasks at a slow rate. 2 g $NaHCO_3$ was added at intervals and the flasks were monitored as before. Sulfate was added to the flasks which had previously contained below 2 % sulfate to bring the sulfate level in them up to that in the 2 % sulfate flask. After the cultures had adjusted to the sulfate, H_2 and decreasing organic nutrients, the contents of two flasks were centrifuged using a Beckman Model J2-21 centrifuge (10 000 rpm for 20 min), the supernatant was discarded and the cells were washed in medium (tap water containing 2 % SO_4^{2-} and 1 % $NaHCO_3$). The suspension was centrifuged again (10 000 rpm for 20 min) and the cells were resuspended in medium. This flask was also monitored.

4.4.2 Results and Discussion

The results showing the adaptation of the cultures to the decline in available organic substrates are depicted graphically in figures 4.12 to 4.16. The flask maintained autotrophically grew slowly as shown in figures 4.17 to 4.21.

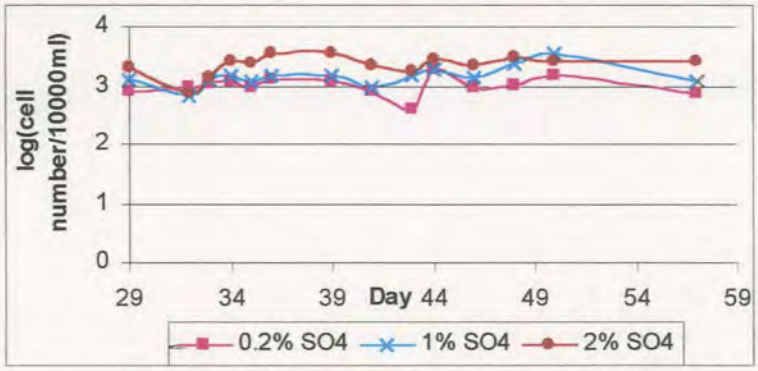

Figure 4.12 Cell Counts as nutrients were depleted

The cell numbers in each flask remained stable even though the organic nutrients were being depleted. This indicates that the cultures were adapting to the changing conditions.

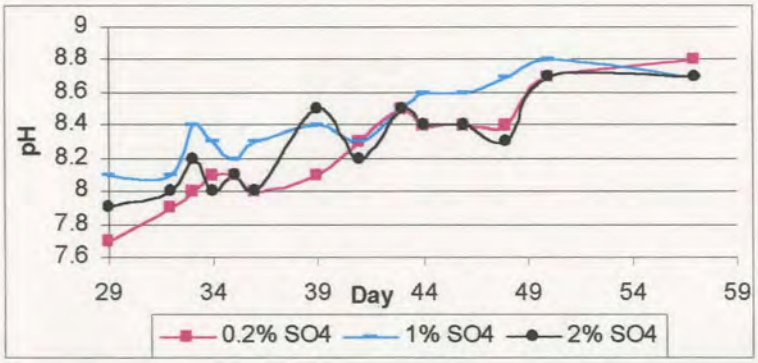

Figure 4.13 pH as nutrients were depleted

The pH of all of the cultures increased in this portion of the study (figure 4.13). This was mainly due to the fact that the hydrogen gas that was bubbled through the flasks acted as a gas purge and removed hydrogen sulfide from the system. To maintain the equilibrium in the solution, more of the dissolved sulfide then became hydrogen sulfide gas and removed hydrogen ions from the liquid phase. The pH therefore increased to 8.7 or 8.8. At this point, the equilibrium would be shifted strongly toward dissolved sulfide and only 2 % would be found as hydrogen sulfide (Hilton and Oleszkiewicz, 1988). Changes in the concentration of dissolved hydrogen ions, the pH, would therefore occur very slowly.

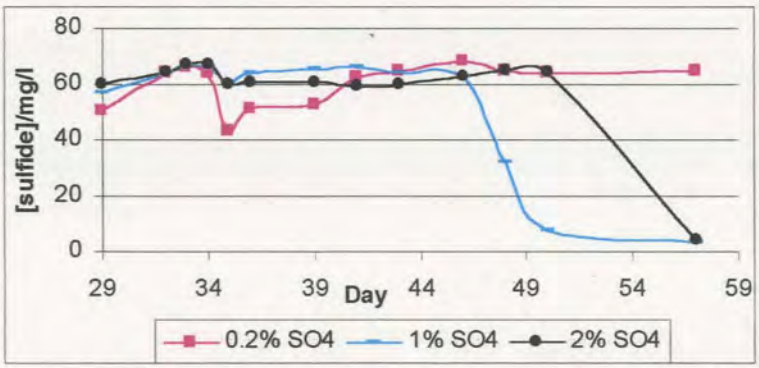

Figure 4.14 Sulfide concentration as nutrients were depleted

The sulfide concentration of the cultures remained the same for over two weeks (figure 4.14). Thereafter, the sulfide concentration in the culture which initially contained 1 % sulfate, began to decrease. Taken in isolation, this could indicate that the SRB were dying, however, the cell counts in the bulk of the culture had not changed and in addition, the culture had taken on a distinctly green colour. The combination of all available information suggested that green sulfur bacteria were proliferating and oxidising much of the sulfide. A similar argument held for the culture initially containing 2 % sulfate except that this culture had a red tinge and purple sulfur bacteria were probably the main oxidisers.

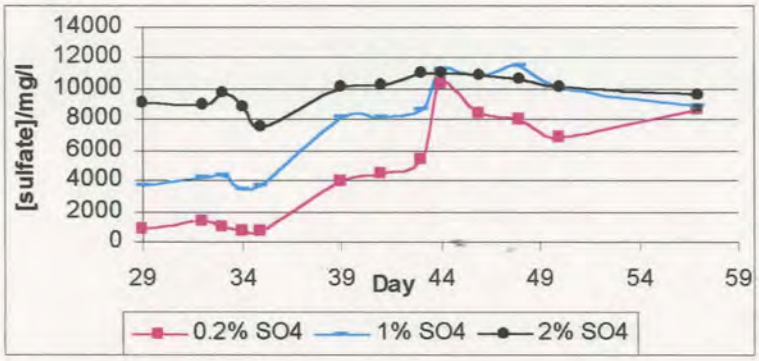

Figure 4.15 Sulfate concentration as nutrients were depleted

As is to be expected, the sulfate concentration in the cultures which initially had lower concentrations, gradually rose as sulfate was added (figure 4.15). In the culture most concentrated with sulfate, as well as in the other cultures between additions, there was a gradual decrease as sulfate was reduced.

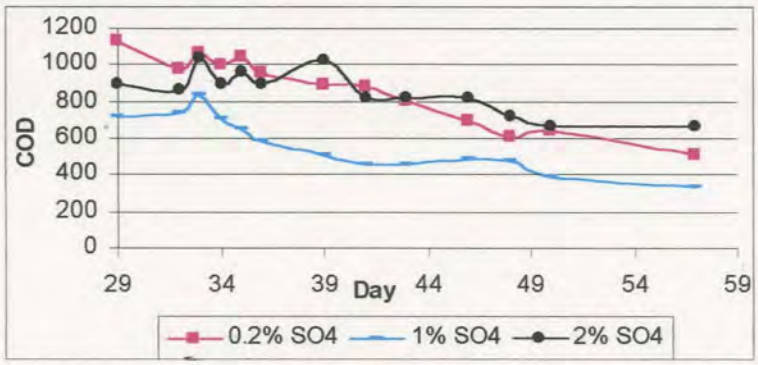

Figure 4.16 Chemical Oxygen Demand as nutrients were depleted

The chemical oxygen demand decreased gradually during the experiment (figure 4.16). Once this had stabilised, it was assumed that at least some of the cells in each culture were growing autotrophically and the bacteria were removed, washed and resuspended in a medium containing only sulfate, carbonate and hydrogen to test for true autotrophic growth.

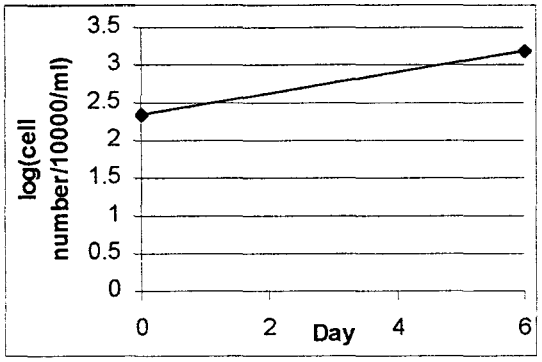

Figure 4.17 Cell count in the autotrophic culture

Cell number increased in the autotrophically grown culture (figure 4.17) even though the COD remained stable at 221 (figure 4.21). This chemical oxygen demand did not reflect the presence of organic nutrients but rather the chemicals in the system (such as sulfate) which also contributed to the oxygen demand. These results indicated that there were either autotrophic SRB in the culture or the growth was a result of a symbiotic relationship between SRB and an autotrophic species.

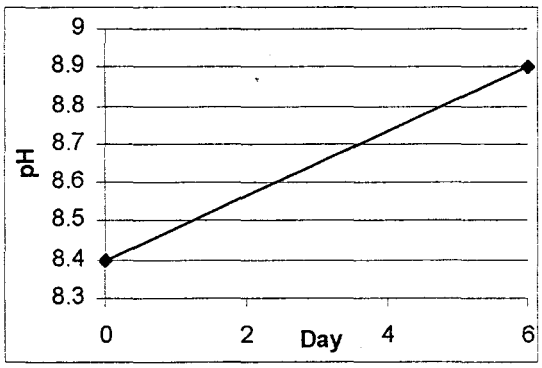

Figure 4.18 pH of the autotrophic culture

The pH of the medium also rose and without the buffering capacity of the yeast extract, the pH was able to rise to 8.9 (figure 4.18).

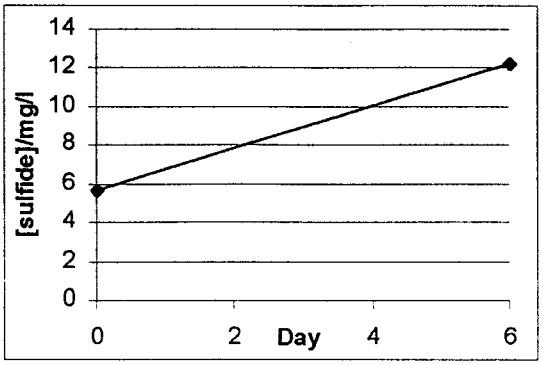

Figure 4.19 Sulfide production in the autotrophic culture

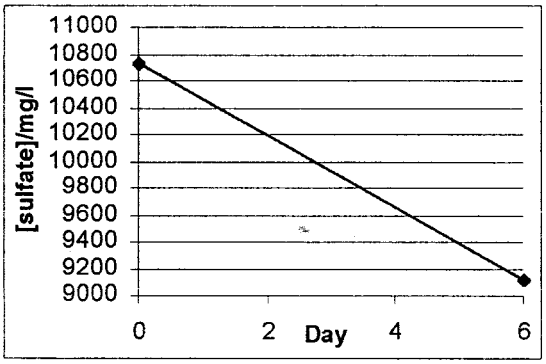

Figure 4.20 Sulfate concentration in the autotrophic culture

Sulfate reduction in the autotrophic culture was indicated by sulfide production (figure 4.19) and a decrease in sulfate concentration (figure 4.20).

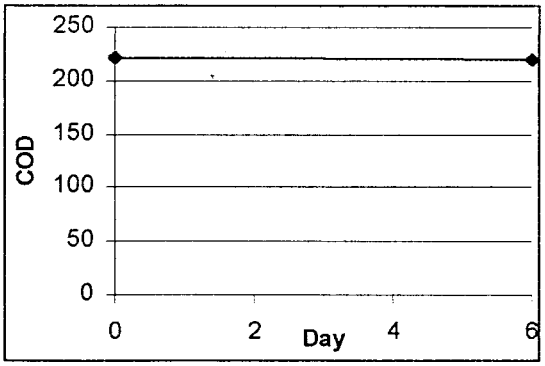

Figure 4.21 Chemical Oxygen Demand of autotrophic culture

The cultures adapted to the decreasing levels of organic nutrients and were finally able to grow in a medium containing only inorganic components. Autotrophic growth of mixed

cultures has been reported before (Van Houten, 1996) using H_2 and CO_2 as a feedstock. Here, sodium hydrogen carbonate has been substituted for the carbon dioxide as the inorganic carbon source because of the effect carbon dioxide has on lime. In a subsequent experiment, calcium carbonate was used as a carbonate source.

By this point, it had been established that a process train involving SRB would be worth investigating. There were two different sites at which sulfate reduction could take place. One was before the carbonation in which case the SRB would need to be able to grow and reduce sulfate at the pH of a $Ca(OH)_2$ solution (around 12.8); and the other was after the carbonation and before the filtration in which case SRB would need to be able to grow and reduce sulfate in a $CaCO_3$ suspension. These two conditions were therefore investigated.

4.5 Sulfate Reduction by SRB at Elevated pH

Determination of the ability of SRB to reduce sulfate at pH levels in excess of pH 8.5

4.5.1 Materials and Methods

At the stage when this investigation was started, the bacteria had made the medium alkaline to a pH of 8.5. Flasks were therefore set up at pH values of 9, 10, 11 and 12. A robust autotrophic population had not yet been established so yeast extract was added as nutrient source.

Anaerobic 500 ml flasks were constructed and filled with 250 ml SRB medium (tap water with 2 % SO_4^{2-} and 0.1 % yeast extract). They were autoclaved and the pH was adjusted. The pH adjustment was initially performed with the supernatant of a precipitator dust suspension but it was found that the pH had risen to 9 by the commencement of the experiment so the pH was adjusted to the specified values with NaOH. Three flasks were studied at each pH. Each flask was inoculated with 25 ml of the culture and incubated on

a rotary shaker at 22°C. The flasks were monitored by counting the cells, determining the sulfate levels and pH.

4.5.2 Results and Discussion

The average changes in cell number, sulfate concentration and pH are shown in figures 4.22 to 4.24.

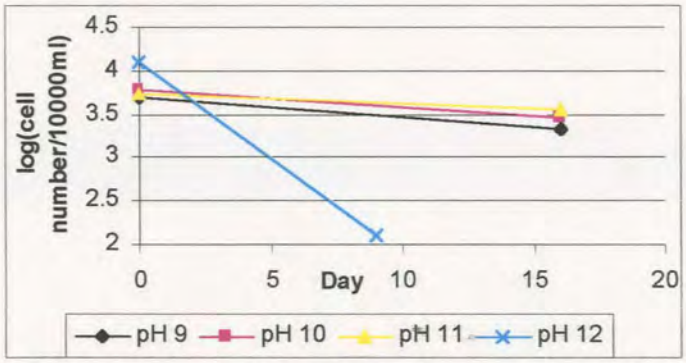

Figure 4.22 Cell counts in cultures at elevated pH

In all cases, the cell count decreased but in the lowest three pH samples, this decrease was slow (Figure 4.22). For the sample at pH 12, decrease was rapid, indicating an inability to survive in the alkaline solution for long. The fact that the cells were able to survive at all is quite remarkable. The optimal pH for growth of most SRB is neutrality (Barnes *et al.*, 1991; Dill *et al.*, 1995, van Houten, 1996) and SRB are usually inhibited at pH values in excess of 9 except when microniches are formed (Widdel, 1988). Under these culture conditions, the cultures slowly deteriorated and if the cells remained in the alkaline solution for an extended time, they would most likely die.

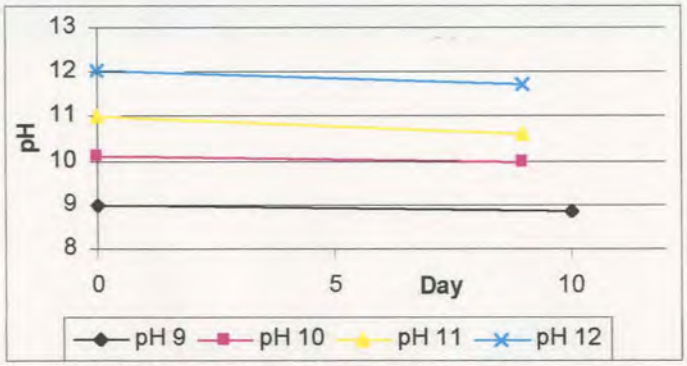

Figure 4.23 pH of cultures at elevated pH

The pH in each culture decreased slightly (Figure 2.23). As has been explained in section 4.2, when hydrogen sulfide cannot escape because of a high pH, the pH of the system may drop as a results of metabolism. This seems to have happened in this case.

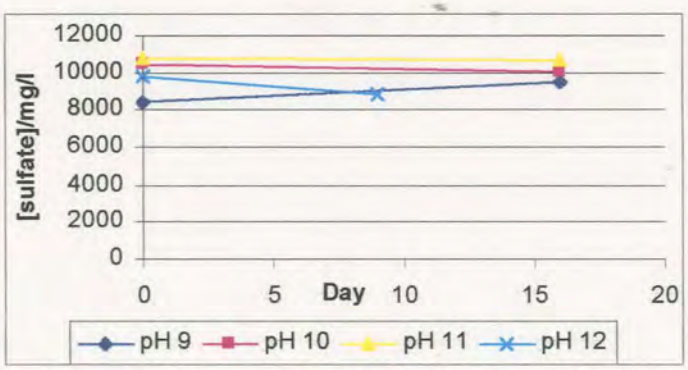

Figure 4.24 Remaining Sulfate in cultures at elevated pH

The sulfate concentration decreased as sulfate reduction took place (figure 4.24). Sulfate reduction should theoretically have been less thermodynamically favourable at high pH (McCartney, DM and Oleszkiewicz, 1993) because at a high pH, almost all sulfide produced remains in solution, eventually giving rise to product inhibition. This may be the reason why the rate of sulfate decrease was fairly slow although this was also linked to the declining cell number.

It has been shown that even though the growth of SRB was inhibited in solutions with a pH greater than 9, sulfate reduction still took place. The SRB culture therefore showed resilience thus far in the experiment and it is likely that if the culture was cycled between

a solution of neutral pH and a lime solution, that the cells would reduce sulfate in the alkaline solution and then replenish their numbers in the optimal solution. Such a hypothesis would certainly be worth further investigation.

4.6 Sulfate Reduction by SRB in a $CaCO_3$ Suspension

Determination of the ability of SRB to reduce sulfate autotrophically in a $CaCO_3$ suspension.

4.6.1 Materials and Methods

Four anaerobic 500 ml flasks were constructed and were filled with 250 ml water and either 2.5 or 5 g $CaCO_3$ (accumulated from the experiments in Chapter 2 and estimated to contain roughly 2% SO_4^{2-}). Duplicate flasks were used at each $CaCO_3$ level. Each flask was inoculated with 25 ml of the autotrophic culture. The flasks were batch-fed H_2 for 10 minutes twice a day while incubated on a rotary shaker at 22°C. The flasks were monitored by counting the cells, determining the sulfate levels and pH.

This experiment was not an easy one to monitor. The flasks were completely turbid and cell counts on the flask material was therefore impossible. Acid was added to dissolve the $CaCO_3$ so that the cells could be seen but this gradually dissolved the cells as well, leading to under estimation of cell number. Different vital stains were tested to find one that would stain the bacteria and not the calcium carbonate crystals but unfortunately, all of the stains were indiscriminate in their colour impartation. Lactophenol blue was eventually chosen as the slight acidity in the solution dissolved the calcium carbonate but did not affect the cells as strongly as the inorganic acids.

Sulfate measurements could not be made using the $BaSO_4$ precipitation method as used in previous experiments as the $CaCO_3$ was insoluble in the analysis conditions and the turbidity imparts masked the turbidity of the precipitated barium sulfate. A Spectroquant

method was therefore used instead (procedure described in Appendix A) as it gave better results.

4.6.2 Results and Discussion

The results are depicted in figures 4.25 to 4.27.

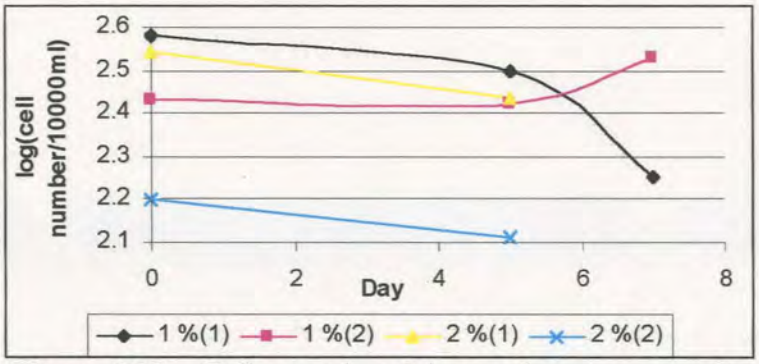

Figure 4.25 Cell count in calcium carbonate suspensions

In three of the four flasks, the cell count dropped as the experiment progressed but as in section 4.5, the decrease was slow (figure 4.25). The high concentration of calcium in the solution may be toxic to the SRB; or to any symbionts necessary for growth of the SRB; or both. In the second case, it would be the lack of nutrients that limited growth of the SRB. Whatever the case, again one could hypothesise that if the cells were kept in the high calcium solution for a time and then transferred to an optimal growth medium to proliferate, that they would survive indefinitely.

In one flask, the cell count increased after 5 days. It may be that some of the cells in the autotrophic culture were resistant to high calcium concentrations and if these were enriched, autotrophic growth in calcium carbonate suspensions could occur.

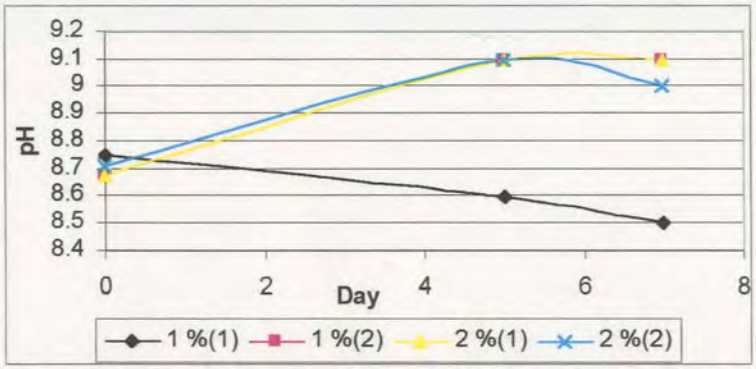

Figure 4.26 pH in calcium carbonate suspensions

In three of the four cultures, the pH rose to 9.1 (figure 4.26). This indicated that sulfate reduction was taking place and that the hydrogen gas was purging the hydrogen sulfide formed, removing hydrogen ions and raising the pH of the solution.

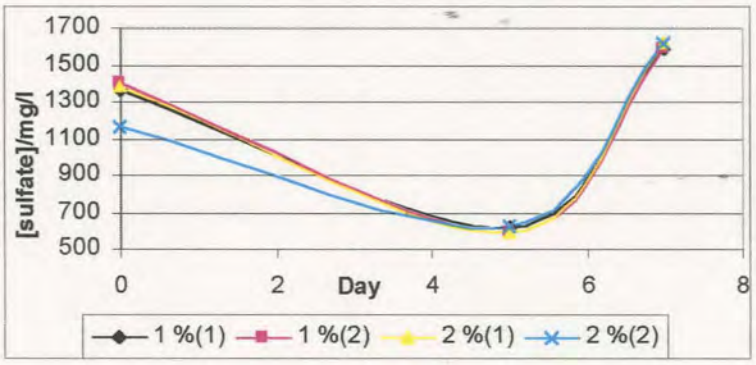

Figure 4.27 Sulfate remaining in calcium carbonate suspensions

The sulfate concentration dropped in all flasks indicating sulfate reduction (figure 4.27). The results for the seventh day indicated an increase in sulfate again to levels higher than the starting concentration. This cast doubt on the accuracy of this method of sulfate determination in these conditions.

Another experiment was then run to monitor the initial situation more closely. Because of the problems experienced with the sulfate measurements, sulfide measurements were used as the primary source of data in this experiment. Sulfide was trapped in 10 ml of a 5

% solution of zinc acetate. An aliquot of this solution was tested using the usual sulfide method.

4.6.3 Materials and Methods

An anaerobic 500 ml flask was constructed and was filled with 250 ml water and 2.5g $CaCO_3$ accumulated from the experiments from Chapter 2 (estimated to contain roughly 2% SO_4^{2-}). The flask was inoculated with 25 ml of the autotrophic culture. H_2 was constantly bubbled through the flask at a slow rate while the flask was incubated on a rotary shaker at 22°C. The zinc acetate in the trap was monitored. When it became cloudy, it was replaced with another trap and a sulfide determination was performed on the zinc acetate suspension. The time was noted and at this time, a sample was taken from the flask and the concentration of sulfate was determined.

4.6.4 Results and Discussion

The sulfide and sulfate measurements are depicted in figures 4.28 and 4.29.

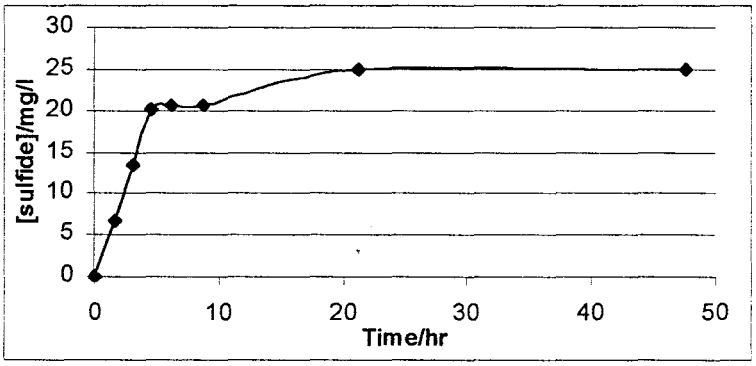

Figure 4.28 Cumulative sulfide production in a calcium carbonate suspension

The sulfide measurements showed that the SRB reduced sulfate almost linearly for the first five hours (figure 2.28). Production was rapid especially considering that only the sulfide found in the gas phase was measured. At a pH of approximately 8.7, this comprised a very small fraction of the total sulfide produced and indicated an active sulfate reducing bacterial population. The sulfide production was slower for the next 15

hours after which no more sulfide was produced. This was probably related to product inhibition.

A cyclic procedure could be carried out in which the SRB are exposed to the suspension for a time, removed and allowed to recover and are later returned to the solution. This initial experiment has suggested an optimal time of 5 hours in the solution.

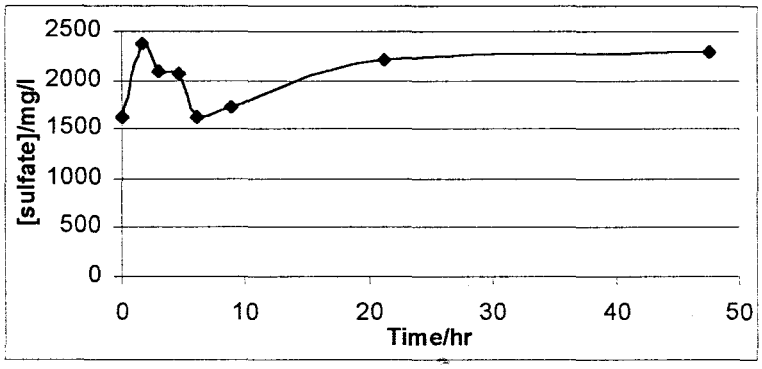

Figure 4.29 Sulfate remaining in calcium carbonate suspension

The sulfate measurements showed fluctuating results, often reporting sulfate values higher than the initial value (figure 2.29). As explained previously, sulfate measurements were not accurate for this system.

4.7 Conclusions

A viable culture of SRB was readily obtained using sewage as a starting inoculum. This SRB population could grow and reduce sulfate in solutions containing between 0.2 and 2 % sulfate. A mixed SRB population could be grown autotrophically although this culture grew slower than a heterotrophic culture and the SRB could survive and reduce sulfate when the pH was between 9 and 12. SRB could reduce sulfate autotrophically in a $CaCO_3$ suspension. The reduction initially occurred quickly and then slowed until no more sulfide was produced.

Chapter 5: Process Development

5.1 Introduction

In the previous chapters, it was demonstrated that white calcium carbonate could be produced by the carbonation of the supernatant of a lime dust suspension. The main impurities present in this calcium carbonate were found to be sulfate and traces of metals. It was shown that the sulfate could be removed by the action of sulfate reducing bacteria and that these bacteria were able to perform this function under the harsh conditions imposed by the system. Sulfide, produced in this case by the bacteria, was also shown to precipitate traces of metals, removing them from the supernatant solution and therefore from the product. The individual components of a process were thus demonstrated and what remained was to incorporate these into a process design; test the feasibility of this system, with modifications if necessary; and finally propose a design for a pilot plant.

It has previously been discussed that producing white calcium carbonate by suspending a source of calcium in water, filtering and then carbonating the supernatant would be uneconomical because of the small yields obtained. The simplicity both in terms of reactor design and reactants made this process a tempting one to follow and it was shown that recycling of starting materials and water was possible. The yield was low but if the volume of the system could be increased without large increases in the cost, the process could be viable. The quarry reactor was therefore proposed.

In this process, waste lime dust would be slaked using Lime Acres water to form a slurry which would be pumped into an empty quarry. Water would enter through a set of pipes with multiple outlets laid at the bottom of the quarry and would percolate through the dust slurry. The overflow would run into a settler with an inlet for sulfide gas. Any solids present in the overflow would settle and any metals would be precipitated as relatively heavy metal sulfide agglomerates. The clarified water would be led into a small carbonation chamber where kiln off-gas is bubbled through it to form a calcium

carbonate suspension. This suspension would run into another quarry where the precipitate would settle. The product would accumulate in the bottom of the quarry and the overflow water would be pumped back into the percolation quarry, thus completing the cycle. When the calcium carbonate had accumulated to a sufficient extent, the inlet stream would be diverted to another quarry allowing the product in the first quarry to dry and to be harvested. The process design is shown in figure 5.1

There are many advantages to this process:

- The water is recycled and the only water additions would be to compensate for evaporation.
- The waste CO_2 from the lime kilns would be utilised.
- The large areas needed for this process are available at Lime Acres.
- The cost of the process is low – the only cost would be the electricity required to drive the pump and the lift between the quarries would not be large.
- The waste dust remains in the quarries and the free lime is removed, eliminating the environmental problems associated with the highly alkaline dust and re-filling the quarries to reclaim the land.
- Large quantities of calcium carbonate could be produced and utilised as white limestone or calcined to give white lime or used as a starting material for processes which would yield high purity calcium products.

Sulfate reducing bacteria can be incorporated into the quarry design to reduce the sulfate impurity to sulfide which would be used in the metal removal stage of the above process. There are two possible locations for the incorporation of the biological system: in the settling step before the carbonation or between the carbonation step and the product settling step. These two possibilities are shown in figures 5.2 and 5.3. It is not anticipated that all material from the quarry reactor will pass through biological circuit but rather a small quantity of material. The sulfate in this material would be removed, leaving calcium carbonate of high purity which would meet the requirements of the pharmaceutical and analytical grade chemical markets.

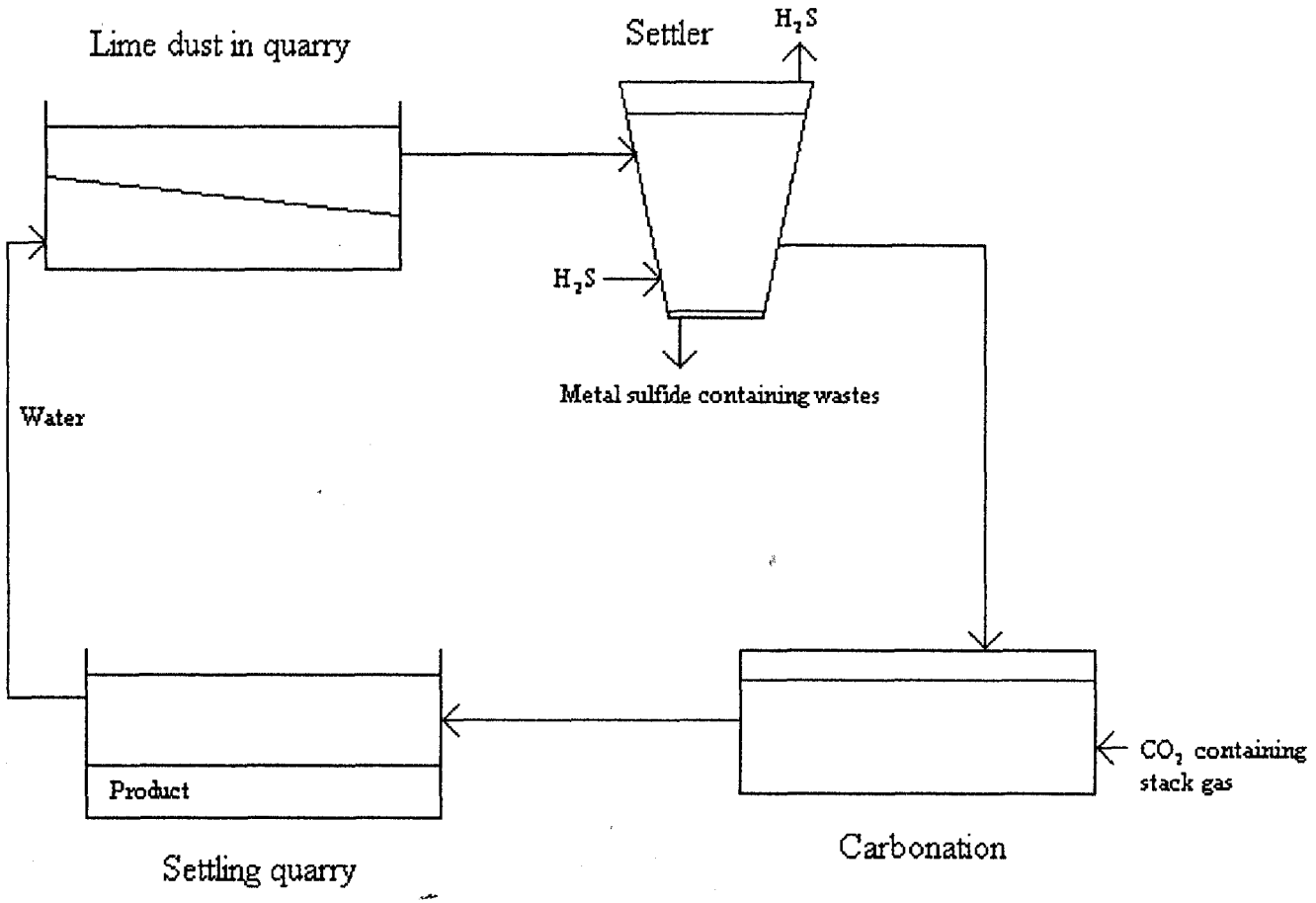

Figure 5.1 Process design for the quarry reactor

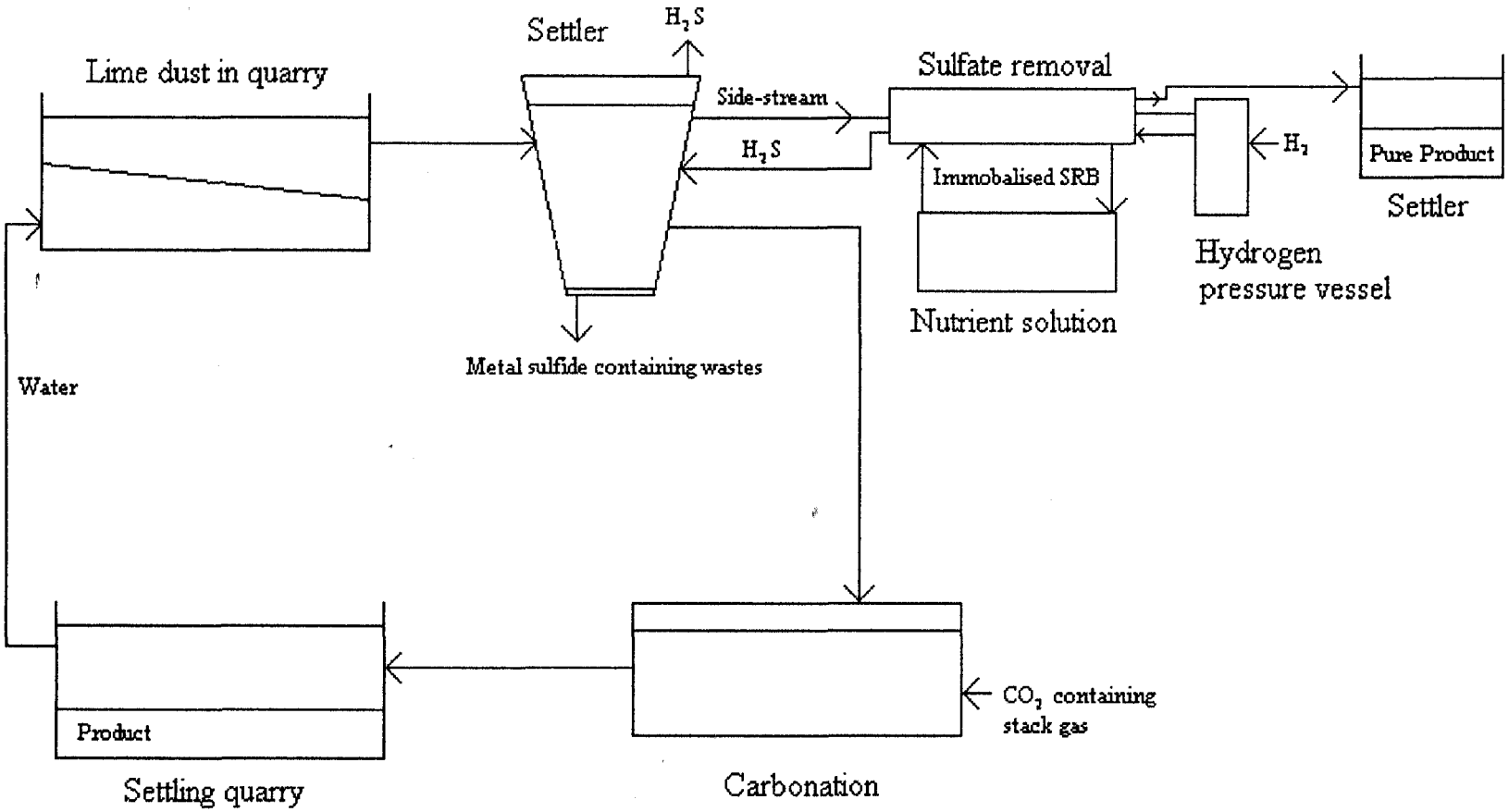

Figure 5.2 Process design for sulfate removal from the lime solution

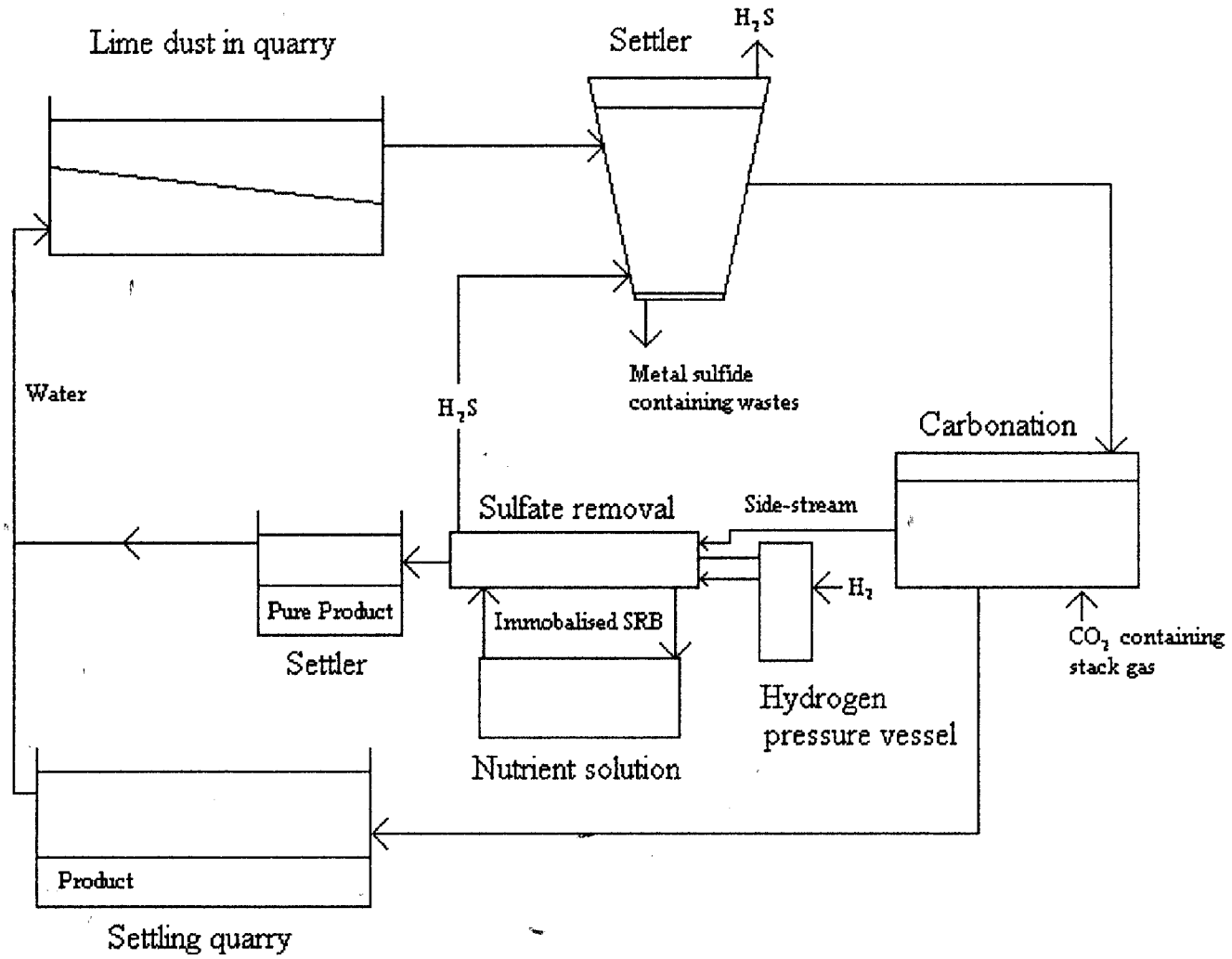

Figure 5.3 Process design for sulfate removal from the calcium carbonate suspension

It was shown in Chapter 4 that while the SRB could reduce sulfate in the absence of an organic carbon source, they did not grow and reproduce rapidly in this medium. A shuttle concept was therefore proposed. In this, the bacteria would grow in an organic medium and then be transferred into the reactor where they would reduce sulfate for a time after which they would be removed and returned to the nutrient medium.

It was proposed that the sulfate reducing bacteria be immobilised on a suitable substrate. It has been reported in the literature that attachment to substrates is possible and SRB have been reported growing on suspended microscope slides (Wijaya *et al.*, 1993), on stone (Maree *et al.*, 1991) and in aggregates with other bacteria (van Houten, 1996). Cell adsorption onto surfaces may also be enhanced by making use of the observation that extracellular polysaccharide production increases if the growth medium is limiting in nitrogen or phosphorus (Okabe *et al.*, 1992). Immobilisation has been seen to improve the process stability (Dill *et al.*, 1995). Microbial systems have also been grown in porous beads (du Plessis *et al.*, 1998) and this may be an ideal support for the SRB in this system as microniches of favourable growth conditions may be formed.

One concern expressed regarding bacterial immobilisation, is disruption of the biofilm owing to shear velocities in the solution (van Houten, 1996). This was worse in the case of hydrogen-fed reactors because of the turbulence caused by the gas phase moving through the liquid phase. To alleviate this, a side-stream of medium was continuously fed through vessel pressurised with hydrogen in order to saturate the liquid and so avoiding introducing the hydrogen as a gas (du Preez and Maree, 1994).

In the shuttle system, the bacteria must be immobilised on a support that would be easily separated from the rest of the system. In the first option (depicted in figure 5.2), the separation of the beads will be fairly simple as the other components of the system at this stage are in the aqueous phase. In the second option (figure 5.3), $CaCO_3$ will be present as a solid but its size and density is vastly different to that of the possible supports and separation should not be difficult.

In order to test the potential of the quarry system described above, a bench-top reactor was constructed to mimic it. This reactor was operated in a continuous fashion, first with minimal supervision as proof of concept and then with constant supervision for 36 hours after which analyses were performed.

5.2 Automatic Bench-top Reactor

An investigation into the feasibility of producing white calcium carbonate using a continuous, unsupervised operation.

5.2.1 Materials and Methods

A 5 l flask served as the water feed reservoir. A tube led from this reservoir, through a peristaltic pump and entered the bottom of the dust-bed container which was half filled with a 50:50 mixture of LK6 cyclone dust and LK9 precipitator dust. The dust was slurried and allowed to cool to room temperature before being poured into the container and allowed to settle. Water was pumped up the dust-bed until it reached the first overflow point. This was only open during the start-up procedure and allowed the calcium saturated water to by-pass the carbonation chamber until the dust-bed had settled and the overflow was clear. The first overflow point was then closed and the water left the dust-bed container and entered the carbonation vessel after passing through a U-tube which served as a settler for sediment carried in the overflow. CO_2 was bubbled through the carbonation vessel to form a very fine $CaCO_3$ precipitate in suspension. This then flowed into a settler which had a volume of about 25 l but was partially separated. The overflow initially entered a central tube through which it moved down into the base of the settler. The $CaCO_3$ was deposited at the bottom of the settler. As the level of water in the settler rose, it reached an overflow point from which it re-entered the feed reservoir. Control of this process was not rigorous and it was operated continuously and unsupervised. There were openings in the tops of the reactors and liquid samples could be taken from each vessel. The pH of samples taken from the dust-bed container, the

carbonation vessel and the overflow to the settler was used periodically to determine the optimal flow rate of the CO_2.

5.2.1.1 Apparatus

A schematic diagram of the apparatus used is shown in figure 5.4 and a photograph of the apparatus is shown in plate 5.1.

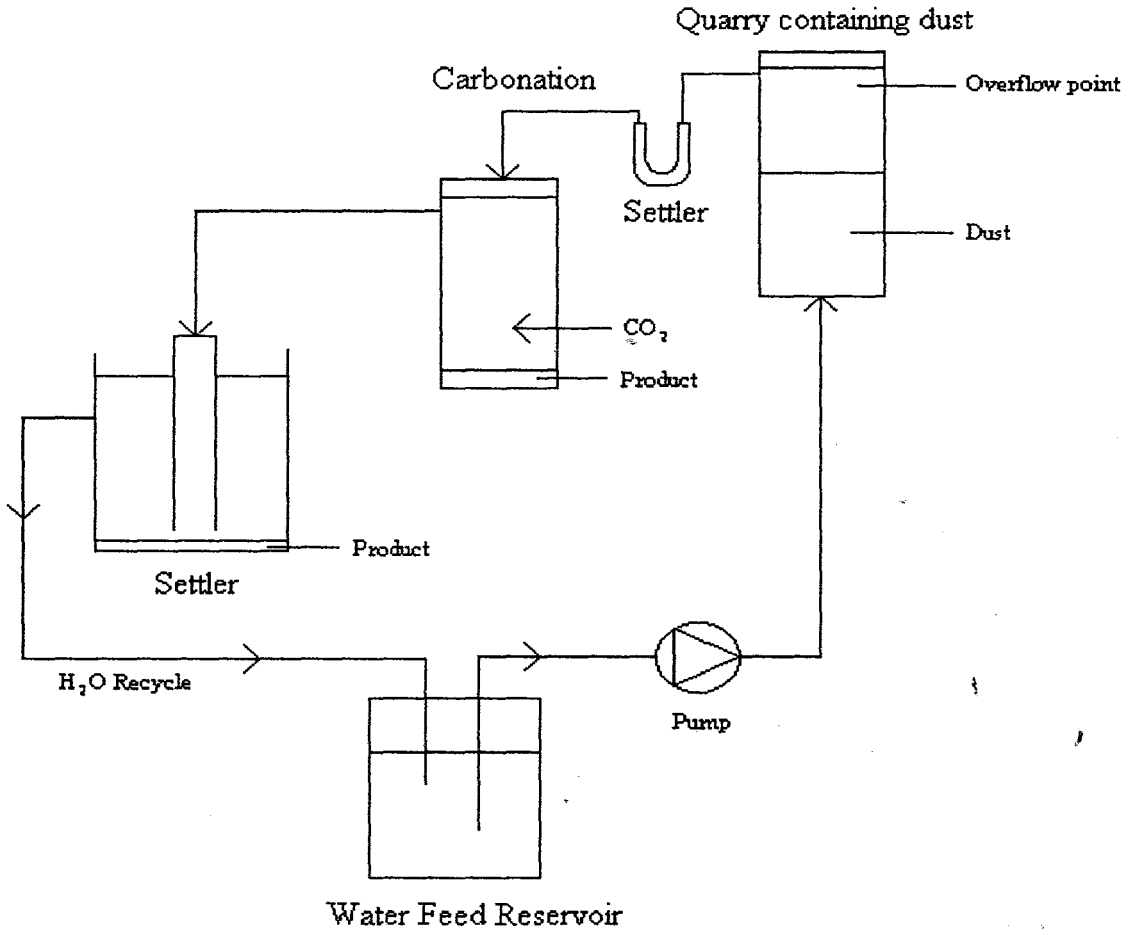

Figure 5.4 Process design of automatic bench-top reactor

5.2.2 Results and Discussion

The reactor was run for over two days and white $CaCO_3$ was deposited at the bottom of the settler. The system was operated continuously without constant supervision or any

form of feedback control. It was seen that continuous operation was possible and the experiment was then repeated under controlled conditions.

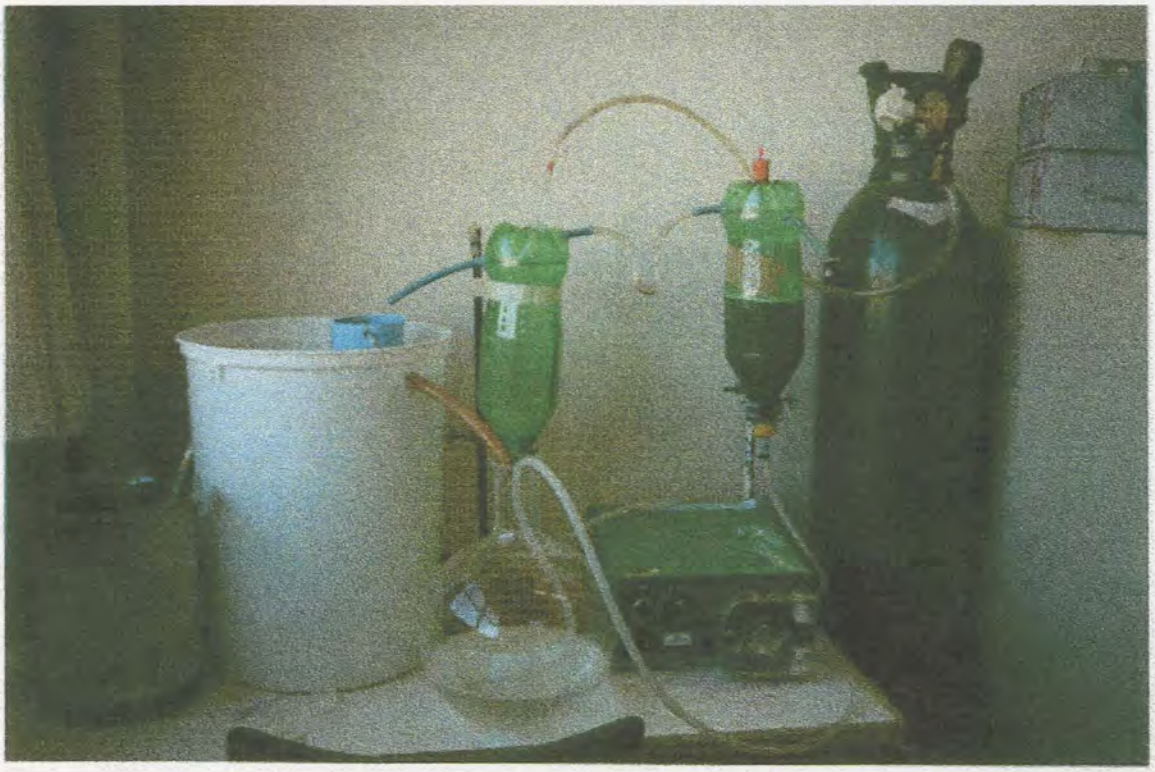

Plate 5.1 Bench-top Reactor

5.3 Monitored Bench-top Reactor

An investigation into the feasibility of producing white calcium carbonate using a continuous, supervised operation and determination of the purity of the product thus obtained.

5.2.2 Materials and Methods

The method followed was the same as in part 5.2.1 except for the addition of an Orion 710 A pH meter in the carbonation vessel (figure 5.5) which allowed the operator to adjust the speed of the pump and the rate of CO_2 addition to maintain the pH at 8.2. Exactly 600 g of starting material was used.

5.2.2.1 Apparatus

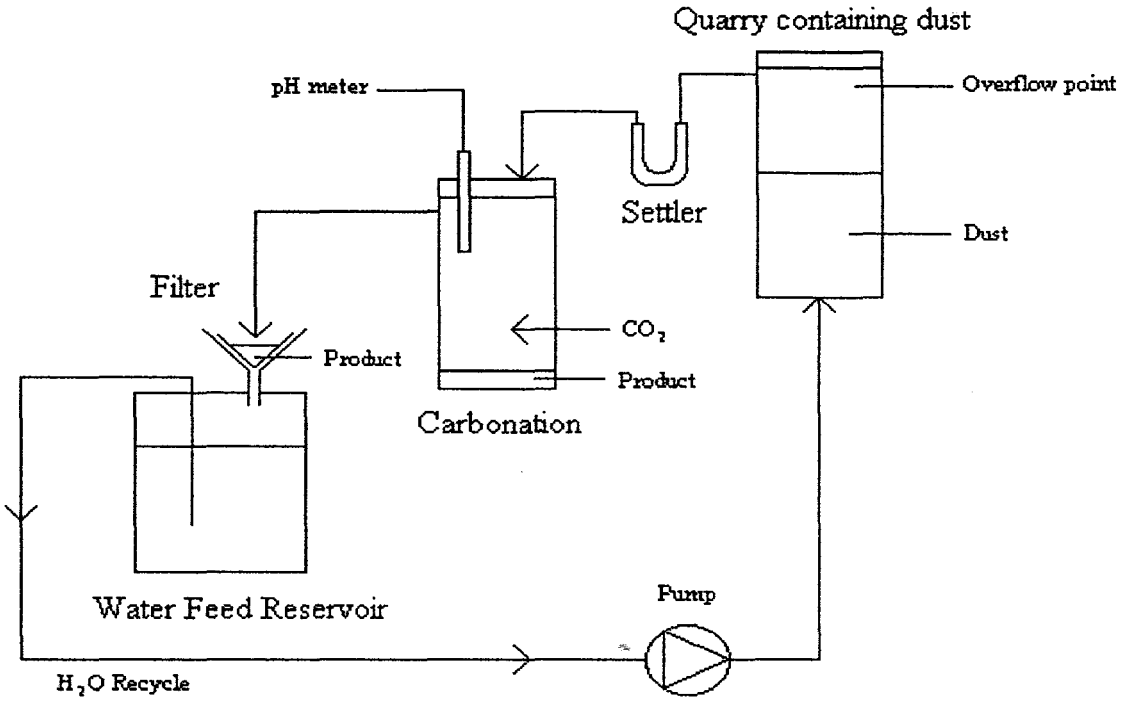

Figure 5.5 Process design for controlled reactor

5.2.2.2 Sampling

Samples of the initial dust sample, the product and the final residual dust were taken.

5.2.2.3 Chemical Analyses

A pressed pill was made of the product, pressed beads were made of the starting and final materials and these were analysed using XRF. Free lime determinations were also performed on the starting and final materials.

5.2.3 Results and Discussion

5.2.3.1 Change in Starting Material

The results of the XRF pressed bead analysis on the starting and final materials are shown in figure 5.6

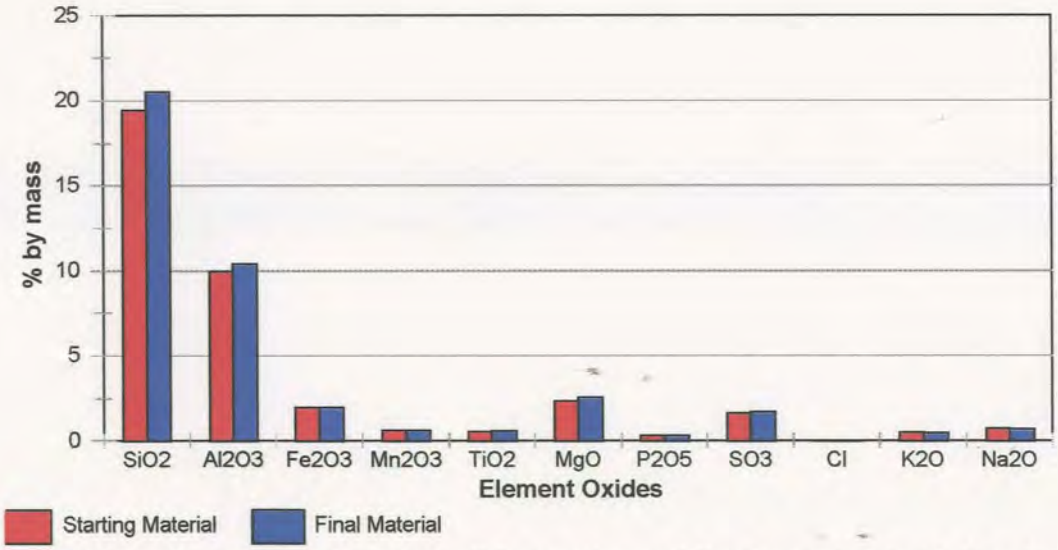

Figure 5.6 Pressed bead analysis of the starting and final materials in the bench-top reactor

As before, the calcium oxide peak has not been shown on the graph as this would overshadow the other results. In this case, the calcium oxide content of the dust material dropped from 66.18 % to 64.94 % which is in keeping with the hypothesis that available lime would be removed from the dust in the first vessel and would be available for precipitation as calcium carbonate in the carbonation vessel. The amount of calcium in the dust material therefore decreased as the process continued. The relative amounts of major impurities like silica, alumina and magnesium increased slightly as they made up a greater proportion of the remaining material. The sulfate concentration, conversely, remained almost constant, as sulfate is sparingly soluble and as the process continued, some dissolved and precipitated with the product. The relative concentration did not increase as it did with the other major impurities. Changes in the minor impurities are slight.

5.2.3.2 Product

The pressed pill analysis of the precipitated calcium carbonate is shown in figure 5.7

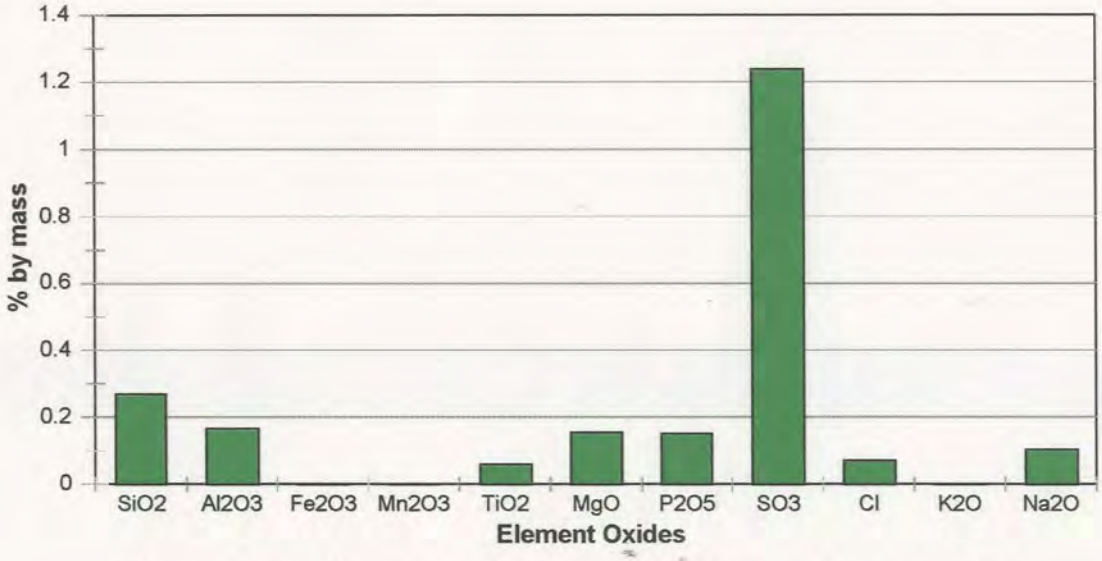

Figure 5.7 Pressed pill analysis of the calcium carbonate product

The results confirmed what had already been shown in the batch, recycling studies, namely that sulfate was found as a major impurity while the other impurities were minor. The sulfate could be removed using sulfate reducing bacteria. Substantial amounts of silica, alumina and other element oxides were found in the product. This was not seen in the batch studies and was therefore attributed to carry-over of low density dust material. This would be minimised or eliminated by the addition of a sulfide settler as proposed. In the bench-top reactor, the settler was simply a small U-tube and it did not prove to be effective enough. An increase in the volume of this settler and addition of sulfide, which would cause the formation of denser metal sulfide agglomerates, would increase the clarity of the water flowing into the carbonation vessel.

It should be noted that no manganese or iron oxide was detected in the product and the calcium carbonate was essentially white. This product would be suitable for many industrial applications requiring white lime but the product would not meet the specifications of applications requiring pure calcium carbonate. For these, the more

rigorous process depicted in figure 5.1 and incorporating the SRB as shown in figure 5.2 or 5.3, would be recommended.

5.2.3.3 Free lime

The results of the free lime analyses are given in Table 5.1

Table 5.1 Free lime determination of the starting and final material in the bench-top reactor

% Free Lime		Yield of $CaCO_3$/g		
Initial	Final	Theoretical	Actual	Left
16.40	9.25	175.59	24.12	99.04

The reactor was operated continuously for 36 hours. The % free lime in the final material showed that operation could have been continued for longer and in an industrial setting, with automatic controls, the process would only be stopped when the lime in the starting material was exhausted.

In general, various points were noted in the construction of this pilot plant and these should be taken into account when embarking on a pilot plant or full scale design.

Firstly, the dust was initially added as a solid and hydrated *in situ*. It was found that the heat generated was substantial and the plastic dust-bed container cracked. In subsequent experiments, the dust was first slurried and allowed to cool before it was added. If the process is performed on a larger scale, the hydration of the dust must be done carefully to avoid the danger of heat generation.

Secondly, in the bench-top reactor, all of the water entered through one opening and the water eventually found the path of least resistance, forming a channel, and exhausted the available lime in this region. The surface area of dust exposed to the water was not optimal. It is therefore recommended that in the scale-up, the water should be introduced

utilising an appropriate diffuse design. If the water is introduced through many smaller holes instead of one large hole, this problem could be minimised.

In terms of capital expenditure, it has been proposed that existing empty quarries be used as the reactors in the process. The expense would therefore be limited to purchase of pipes and a pump as well as purchase or construction of carbonation and settler vessels and of the SRB shuttle circuit. Running costs for the chemical system would be limited to the energy required to drive the pump. In the biological circuit, the bacteria would reduce sulfate using H_2 and dissolved carbonate ions. The H_2 would come from producer gas that could be made on site at Lime Acres, from coal. This would add the cost of a producer gas plant to the capital expenditure but the expenses thereafter would be minimal as the feed stock, coal, is available at Lime Acres.

It has been proposed that the SRB would then be shuttled between the sulfate solution and a nutrient rich solution. In these experiments, the organic nutrient used was yeast extract, however, this is likely to be substituted in the plant process to lower running costs. Possible substitutes are sewage, which has been shown in this study to be a suitable nutrient source, or an extract of *Spirulina*, which has been grown in water available at Lime Acres with minimal added nutrients.

5.4 Conclusions

A process was developed that brought calcium into solution and allowed that calcium to be precipitated in bulk as white calcium carbonate. This process could be operated automatically and unsupervised or with feedback control mechanisms in place. Carry over from the starting materials contaminated the product and a design was therefore proposed which integrated the chemical and biological facets of the work. This process would eliminate any residual dissolved metals and allow settling of less dense solid particles making the calcium carbonate produced suitable for high-grade applications.

Chapter 6: General Discussion

White calcium carbonate is a product with many uses. Currently, there are no local suppliers of this material and the South African market is supplied by imported white lime. An opportunity exists for the local manufacture and sale of this product.

White calcium carbonate has been manufactured in large quantities for over a century and many methods are available for this manufacture (elaborated in Chapter 1). The simplest method of preparation is the carbonation (using carbon dioxide gas) of a calcium hydroxide suspension or solution. Calcium carbonate is formed and because of its low solubility in water, the resulting fine, white precipitate is easy to harvest. Product formed in this way usually has a high level of purity as the impurities present in calcined limestone (from which calcium hydroxide is made) are found in highly oxidised forms. These oxides have low solubilities in water. A filtered solution of calcium hydroxide is therefore inherently fairly pure and the product of the carbonation of this filtrate retains this purity.

There are disadvantages to the technique proposed, however, the greatest of these being the low solubility of calcium hydroxide in water. For this reason, many methods of production make use of chemicals that enhance the solubility of calcium hydroxide in water (elaborated in section 1.2.3). The drawback of this approach is that some impurities commonly found in calcium hydroxide are soluble under the reaction conditions. The simplicity of the method is also surrendered as more sophisticated reactors and control mechanisms become necessary.

A simple carbonation of a calcium hydroxide solution was chosen as the starting point for this study. The Pretoria Portland Cement, Lime Division factory at Lime Acres in the Northern Cape, supplied the calcium materials used. An investigation of these materials showed that they contained various impurities, the most notable of these in terms of potential for impartation of colour to the calcium carbonate product, were iron and

manganese. Oxides of these elements are only sparingly soluble in water and it was possible to remove traces through precipitation with sulfide ions. When the calcium hydroxide was completely dissolved, using concentrated inorganic acids, subsequent precipitation of iron and manganese with hydroxide ions was not successful. While most of the iron and some of the manganese was removed, substantial amounts (easily detected even by methods with low sensitivity) remained in solution and contaminated the calcium carbonate product. In addition, much of the calcium which was dissolved in the acid solutions, was precipitated as calcium hydroxide during the hydroxide precipitation and the corresponding yields of calcium carbonate were low.

While calcium hydroxide has been the usual starting material for calcium carbonate preparation by the method outlined above, some workers have used waste calcium oxide-containing dusts as the calcium source (McAllister, 1952; Gavrilescu, 1964; Laine *et al.*, 1981; Gospodaru *et al.*, 1981; Todaka *et al.*, 1988a; 1988b; Nakajima and Saito, 1993; Li, 1994; Huege, 1997; Shibazaki *et al.*, 1995). The effect of using this waste dust on the purity of the product was found to be minimal with most of the impurities remaining in the insoluble fraction. This implies that waste lime dust can be utilised in this process. The cost of the manufacture is thereby decreased and in addition, an unpleasant and potentially hazardous material is rendered harmless. The free lime from the material is removed and the remainder is suitable for landfill.

Recycling of the water and starting material used in the process served to increase, rather than decrease, the purity of the calcium carbonate product. This is important for water conservation as water is not consumed in the process but merely utilised. The only water losses predicted would be those resulting from evaporation, a process that would be equally evident in the quarries where water is currently held at Lime Acres.

The product of the recycling process was a white limestone and would be suitable for many applications. It was not a chemically pure calcium carbonate, however, and did have some impurities. The major impurity was sulfate and the amount was dependent upon the starting material used. It was found that the portion of sulfate found in the

product decreased as the material was recycled and sulfate levels in the starting material decreased. For industrial use, sulfate levels of 2 % and under (by mass) may not be significant but certainly for high-grade applications such as pharmaceutical and analytical chemical grade calcium chemicals, this level of sulfate would need to be decreased.

There are many possible sulfate removal techniques which could be applied here but the use of sulfate reducing bacteria to reduce the sulfate to sulfide ions, had never been applied to calcium carbonate purification previously and this option was investigated. A mixed SRB population was isolated and studied. These bacteria were highly active at sulfate concentrations up to 2 % and were capable of autotrophic growth. It was seen that they could reduce sulfate in solutions with elevated pH and in calcium carbonate suspensions. They did not grow readily in the latter media, however. Still, the versatility of the robust SRB population provided many options for process design.

Owing to the low solubility of calcium hydroxide and the corresponding low yields of product obtained from a single cycle, a process was sought which would accommodate large volumes simply and inexpensively, to overcome the low yields. The possibility of converting two empty lime quarries into a reactor series was proposed. In this design, lime dust would be perfused with water which would overflow into a settling vessel. In this vessel, the solution would make contact with sulfide ions and residual metals would form insoluble metal sulfide agglomerates. These agglomerates would incorporate other impurities and settle out of solution (Watson *et al.*, 1995). The overflow would pass into a carbonation vessel where CO_2 would be added continuously so that the pH of the overflow from this vessel would be no lower than 8.2. The calcium carbonate formed in the carbonation would settle in another quarry until there was sufficient material to harvest, and the overflow water would be pumped into the first quarry to complete the cycle. Harvesting the product would be a simple procedure of draining the excess water from the quarry and allowing the product to dry and removing it using front-end loaders.

The bulk product would contain sulfate as an impurity. Another minor circuit was therefore proposed to manufacture smaller quantities of high-purity calcium carbonate.

This would involve removing liquid from either the carbonation vessel or the settler and treating this with SRB. The SRB would be immobilised on a support and would be shuttled between the sulfate containing solution and a nutrient solution. The sulfate impurity in the calcium solution or suspension would be reduced to sulfide gas which would be directed into the settler in the bulk process. Once the sulfate had been removed, the purified calcium-containing liquid would be processed in a similar fashion to that already discussed.

The result of the processes discussed above would be a large tonnage of white limestone and a smaller mass of high-purity calcium carbonate. Both have immediate markets and in addition, it would be possible to use the high-purity calcium carbonate as a starting material for other pure calcium products. For this, the calcium carbonate would be calcined and dissolved in water. The desired anion, produced either chemically or biologically, would then be added and calcium products such as calcium oxalate or calcium citrate would be formed. These compounds have a high value and would bring further revenue.

Overall, a process has been proposed, and tested at a bench-top scale, for the manufacture of bulk quantities of white lime. Further modifications have been suggested which would allow the concomitant production of smaller amounts of high-purity calcium carbonate using a biological system to complement the chemical process. The basic components of this system have also been tested and proved favourable. The financial demands of the process are small in terms of capital expenditure as many reactors are already in place and the running costs will be minimal. The profit is essentially the selling price of white lime (R 600 per tonne), pure calcium carbonate (R 3000 per tonne) and possibly other high-grade calcium chemicals which would favourable for an industry where the main products sell at between R 200 and R 400 per tonne.

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Appendix A: General Methods

A.1 Preparation of a Pressed Pill

1. Wax was placed in an aluminium cup until it was about three-quarters full
2. As all samples already had fine particle size, they were not ground
3. Cup was filled with sample
4. This was pressed into pill form using a Hertzog press (Type HTP 40)

A.2 Preparation of a Pressed Bead

1. Sample was ground using a Siemens ring mill until fine.
2. Sample (2g) was weighed into a platinum crucible
3. This was ignited at 1000°C for 60 minutes and cooled for 30 min in a dessicator
4. Sample was weighed and the Loss on Ignition (LOI) was determined
5. Loss-free sample (1.2 g) was weighed into a platinum crucible and 6.0 g Spectroflux was added
6. This was ignited at 1050°C for 5 min
7. It was remove from the furnace, swirled and returned to the furnace for 5 min
8. Sample was removed, swirled and a spatula tip of caesium iodide was added with swirling
9. Sample was returned to the furnace for 5 min
10. Sample was removed, swirled and pour into a Scientec Glassbead press maintained at 350°C
11. Molten bead was pressed with the plunger
12. Plunger was removed and the mould was covered with a cap
13. Apparatus was inverted, the mould was removed and the bead was covered with another cap
14. Bead was left on the heating block until all beads had been prepared
15. Heating block was switched off and the beads were allowed to cool and anneal slowly

A.3 Spectrophotometric Analysis for Sulfide

1. $FeCl_3$ solution (0.5 ml) (8 g $FeCl_3$ in 500 ml of 6 M HCl) was transferred to test-tube using an autopipette
2. *N,N*-dimethyl-*p*-phenylene diamine dihydrochloride solution (0.5 ml) (0.4 g *N,N*-dimethyl-*p*-phenylene diamine dihydrochloride in 100 ml 6M HCl) was added
3. 4 ml calibration standard or sample (diluted if necessary) was added
4. Solution was mixed and left for at least 60 min for colour development
5. Sample was read at 670 nm on a Unicam Heλios α Spectorphotometer
6. Recommended calibration standards were: 0.2, 0.4, 0.6, 0.8 and 1.0 $mg.l^{-1}$

A.4 Spectrophotometric Analysis for Sulfate

1. Buffer solution A: 30g $MgCl_2$; 5 g sodium acetate; 1g KNO_3; 20 ml acetic acid made up to 1 l with milli-Q water was prepared
2. Using an autopipette, 0.2 ml sample was transferred into a test-tube
3. Milli-Q water (4.8 ml) was added followed by buffer solution A (1 ml)
4. Barium chloride solution (0.5 ml) (40g/l) was added
5. Sample was mixed on a vortex mixer for 60 seconds
6. The absorbance at 420 nm was read on a Unicam Heλios α Spectrophotometer
7. The blank used had no barium chloride added
8. The absorbance reading was converted into a sulfate concentration using:

Sulfate = (291.99 x Absorbance – 4.82) x 25 (dilution factor)

A.5 Spectroquant Sulfate Determination

1. Sample (diluted if necessary) (2.5 ml) was pipetted into a test-tube
2. Solution SO_4-1A (2 drops) was added
3. SO_4-2A (1 green microscoop) was added and the tube was well mixed

4. The tubes were left to stand for 5 min in a water bath set at 40^0C and were well shaken during this time.
5. SO_4-3A (2.5 ml) was added using the syringe supplied
6. The suspension was mixed and filtered
7. SO_4-4A (4 drops) were added, the solution was mixed and incubated at 40^0C for a further 7 minutes.
8. The absorbance relative to a blank was read on a Merck SQ 118 spectrophotometer (program 121).

A.6 Cell counts

A haemocytometer is a glass slide containing two small counting cavities. Parallel lines are scored into these cavities to give a 5 x 5 grid. The slide is calibrated so that once the cover slip is in place, the cells covering the 5 x 5 grid are those found in 10^{-4} ml of the original sample. The cells in the four corners of the grid and the center block are counted and that number is multiplied by 5 to give the total number of cells in 10^{-4} ml.

1. A drop of sample was placed on the haemocytometer and the coverslip was lowered into place
2. The cells reasonably expected to be SRB were counted

A.7 Chemical Oxygen Demand

1. Sample (4.0 ml) (diluted if necessary) was transferred into a COD tube using an autopipette.
2. Merck COD solution A (0.3 ml) was added, followed by Merck COD solution B (2.3 ml).
3. The tube was capped, mixed and heated at 148°C on a Merck TR 205 heating block for 2 hrs, removed and allowed to cool to room temperature.
4. The absorbance was measured relative to the blank using a Merck SQ 118 spectrophotometer (program 29).