Development of a family of large-scale biotechnological processes to desulphurise industrial gasses

Albert J.H. Janssen[1,3], Robin van Leerdam[1], Pim van den Bosch[1], Erik van Zessen[2], Gijs van Heeringen[3] and Cees Buisman[1]

[1] *Sub-department of Environmental Technology, Wageningen University, Bomenweg 2, P.O. Box 8129, 6700 EV Wageningen, The Netherlands*

[2] *Paques B.V., T. de Boerstraat 24, 8501 AB Balk, The Netherlands*

[3] *Shell Global Solutions Int. B.V., Badhuisweg 3, 1031 CM Amsterdam, The Netherlands*

ABSTRACT

In this paper an overview is given of a new biotechnological process to remove hydrogen sulphide from gas streams. This process is jointly developed by Wageningen University, Delft University of Technology, Paques B.V. and Shell Global Solutions International B.V. In 1992, the first full-scale installation for H_2S removal from biogas was taken into operation whilst in 2002 the first unit for high pressure natural gas desulphurisation was started-up. The removal of sulphur dioxide from flue gasses is feasible as well and in 2006 the first unit went on-stream in China. Currently, more than 75 full-scale plants are in operation worldwide. The formed bio-sulphur has a hydrophilic nature which enables its re-use, *e.g.* as a fertilizer or fungicide.

1 RELEASE OF SULPHUR-CONTAINING GASSES INTO THE ATMOSPHERE

The increase in global population from 6 billion people in 2005 towards an expected number of 9 billion in 2050 is inevitably associated with a continued industrialization, urbanization and motorization. Without doubt, this will lead to a strong growth of the need for energy, especially in countries such as India and China that are on the eve of an industrial revolution. According to the International Energy Agency the energy consumption will increase with some 60 per cent in 2030 whilst in 2050 the global

energy consumption is expected to be three times higher than today. In the past, increased pollution effects were recorded in both industrial regions and on a global scale due to an increase in industrial activities (Earthwatch United Nations Environment Program, 1992). Some of these environmental problems were related to the emission of sulphur compounds, mainly as sulphur dioxide (SO_2) that is formed from the combustion of sulphur containing fuel sources. For example, during a period of dense fog in the winter of 1952 more than 12,000 casualties were recorded in London as a result of the emission of SO_2-containing flue gasses. Even today fossil fuel combustion accounts for approximately 90 per cent of the global man-made emission of SO_2. Other major sources of SO_2 are the processing of sulphide ores, oil refining and sulphuric acid production (Brimblecombe *et al.*, 1989). In 1989, the global anthropogenic emission of sulphur to the atmosphere was estimated at 93 to over 200 million tons per year whereas over 90 per cent of all anthropogenic emissions of SO_2 occur in the northern hemisphere (Aneja, 1990; Brimblecombe *et al.*, 1989; Dignon and Hameed, 1989). In tropical regions natural emissions from soils, plants, the burning of biomass and volcanoes are believed to be the predominant sources of SO_2 (UNEP, 1991). When released to the atmosphere, sulphur dioxide is a severe acidifying compound causing acid-rain.

In case hydrogen sulphide is present in hydrocarbon containing gas streams, such as landfill-/biogas or natural gas, it has to be removed before incineration of the hydrocarbon stream because of its toxicity, corrosivity and bad smell. Emission of hydrogen sulphide into the atmosphere is therefore mainly a result of volcanic activities and evaporation from oceanic waters (Brimblecombe *et al.*, 1989). In the atmosphere hydrogen sulphide causes acid rain due to its reaction with ozone to sulphuric acid.

Fortunately, since the 1970s in many industrialized nations SO_2 levels declined as a result of various emission control strategies such as selection of fuels with a low sulphur content, specialized combustion processes, coal desulphurisation and the development of new gas treatment processes. The main end-products from these processes are elemental sulphur and gypsum for respectively H_2S and SO_2 removal.

2 EXISTING REMOVAL METHODS FOR H_2S REMOVAL

2.1 EXISTING REMOVAL METHODS FOR H_2S

Hydrogen sulphide (H_2S) is a weak acid which dissociates into HS^- (pK_1=7.04) and S^{2-} (pK_2=11.96) with pK values at 18°C (Lide, 1995). The term «sulphide» is applied for all three entities. Removal of hydrogen sulphide from gases or wastewaters is required for reasons of health, safety and corrosion. The toxicity of hydrogen sulphide gas is well documented (Table 1). In the USA the worker exposure limits are 10 ppm (14 mg/m^3) TWA (time weighted average) and 15 ppm (21 mg/m^3) SEL (single exposure

limit). Hydrogen sulphide becomes progressively more dangerous as the H_2S level incurs above toxic limits (70 ppm), becoming lethal at 600 ppm. Because of its detrimental characteristics, it is forbidden to release sulphide containing effluents and gaseous streams into the environment.

Table 1.
Hazard levels associated with releases of H_2S.

H_2S concentration	**Effects**
1 ppm	Rotten egg smell, odor complaints
10 ppm	Occupational exposure limit for 8 hours
20 ppm	Self-contained breathing apparatus required
100 ppm	May cause headaches/nausea, sense of smell lost in 2-15 minutes
200 ppm	Rapid loss of smell, burning eyes and throat
500 ppm	Loss of reasoning and balance, respiratory distress in 200 minutes
700 ppm	Immediate unconsciousness, seizures
	Without immediate resuscitation breathing will stop, leading to death

For the removal of H_2S from gas streams, various well-established physico-chemical techniques are available. Many of the processes that are in use at present may be grouped into the categories listed in Table 2.

Liquid phase processes are used to remove H_2S from sour gas streams, e.g. natural gas or refinery gas, via absorption down to levels that are typically around 4-10 ppm(v). As a result of steam addition to the loaded solvent, the extracted sulphide is released resulting in concentrated H_2S streams (sometimes called acid gas). Obviously a second step is required to convert this into harmless elemental sulphur (S_8). For H_2S quantities above 20 tonnes per day, the Claus process is commonly applied whilst at low H_2S loads (< 1 tonne per day) the acid gas is often sent to a flare to burn the H_2S to sulphur dioxide. In the Claus process, 1/3 of the H_2S is burned to SO_2 whereafter the remainder reacts with the formed SO_2 to elemental sulphur and water, according to: $2\ H_2S + SO_2 \Leftrightarrow 3\ S° + 2\ H_2O$. As this is an equilibrium reaction, it is not possible to reach a full (*i.e.* 100%) H_2S removal efficiency but in a line-up that comprises 3 consecutive process steps an overall efficiency of 96% is possible. In order to reach more than 99% H_2S conversion a post treatment step, such as the SCOT or Superclaus process is required. Obviously, this will lead to a significant increase in the treatment costs (both capital and operating costs). In the range of 0.1-20 tonnes H_2S per day, direct conversion with chelated iron (ferric) is carried out as in the Lo-Cat and SulFerox process. However, many of these units are plagued by plugging and foaming problems. Therefore the Gas Technology Institute (GTI, Des Plaines USA) concluded that no

existing technology was suitable for treating high pressure natural gas in the medium-scale range (O'Brien *et al*., 2007). Also the popularity of the Stretford process vanished as it became difficult to dispose of sulphur containing vanadium salts, a heavy metal that makes the sulphur product a hazardous waste. For low H_2S loads, i.e. less than 0.1 tonnes per day, caustic scrubbing is still applied resulting in the formation of sulphide rich caustic streams that are quite difficult to dispose. These streams are regularly fed to biological wastewater treatment installations where they stimulate the growth of poor settling filamentous bacteria. Alternatively, iron sponge beds are used for H_2S adsorption. However, due to the heat of reaction and the pyrophoric nature of the formed FeS considerable safety measures have to be taken.

Table 2.
Some examples of physico-chemical processes available for treating sour gases (Jensen and Webb, 1995).

Category	**Example**	**Reagents**	**Products**
Liquid phase chemical reaction	Amines	Alkanolamine	H_2S and CO_2
	Alkaline salts	Potassium carbonate	H_2S and CO_2
Liquid phase physical absorption	Sulfinol	Sulfolane and diisopropanolamine	H_2S and CO_2
	Selexol	Dimethyl ether of polyethylene glycol	H_2S and CO_2
Dry bed adsorption	Iron sponge	Iron oxide	S°
	Molecular sieve	Crystalline alkali-metal aluminosilicates	S°
Direct conversion	Stretford	Sodium carbonate, sodium vanadate, anthraquinone	S°
	Lo-Cat/SulFerox	Iron complexes	S°
	Claus	H_2S and SO_2, catalyst	S°
	SCOT	Cobalt/molybdenum catalyst and alkanolamine solvent	H_2S
	Superclaus	catalyst	S°
	Incineration	Air-oxygen	SO_2

3 NEW PROCESS FOR THE TREATMENT OF H_2S CONTAINING GAS STREAMS

3.1 Introduction

Most important disadvantages attached to existing processes for H_2S and SO_2 removal are:

- High investment costs make the existing process for H_2S conversion only feasible for large-scale applications (Claus process).
- High maintenance costs and downtime are encountered due to foaming and plugging problems (Lo-Cat, SulFerox).
- An expensive post treatment method is needed for more than 99+ H_2S removal efficiencies.
- High energy costs for pumping of limestone-gypsum slurry (SO_2 removal).

As an alternative to the above mentioned existing technologies also microbiological processes for gas treatment are being considered. As these proceed around ambient temperatures and atmospheric pressure, the need for heat, cooling and pressurization power are not needed and thereby cut the energy costs to a minimum. One of the oldest and most commonly found applications of biological gas treatment involves the vent-air treatment at wastewater treatment plants. Worldwide more than 15,000 of these systems are in operation of which most belong to the 'biotrickling' and 'biofilter' type (Van Groenestijn, 2005).

3.2 The biological sulphur cycle

Besides the Carbon- and Nitrogen cycle, the Sulphur cycle is important in nature. It has an oxidative and a reductive side which in a natural ecosystem should be in balance. On the reductive side, sulphate and sulphur function as an electron acceptor in the metabolic pathways, used by a wide range of anaerobic bacteria (Widdel, 1980). On the oxidative side of the cycle, reduced sulphur compounds serve as electron donors for anaerobic phototrophic bacteria or provide growth energy for the colorless sulphur bacteria (Robertson and Kuenen, 1991). The biological sulphur cycle is presented in Figure 1.

On the oxidative side two different biotechnological processes can be distinguished for the removal of hydrogen sulphide. Firstly, genera of the family *Chlorobiaceae* and *Chromatiaceae* catalyze under anaerobic conditions, the photosynthetic Van Niel reaction (Niel van, 1932):

$$2n\ H_2S + n\ CO_2 \xrightarrow{h\nu} 2n\ S^\circ + (CH_2O)_n + n\ H_2O$$

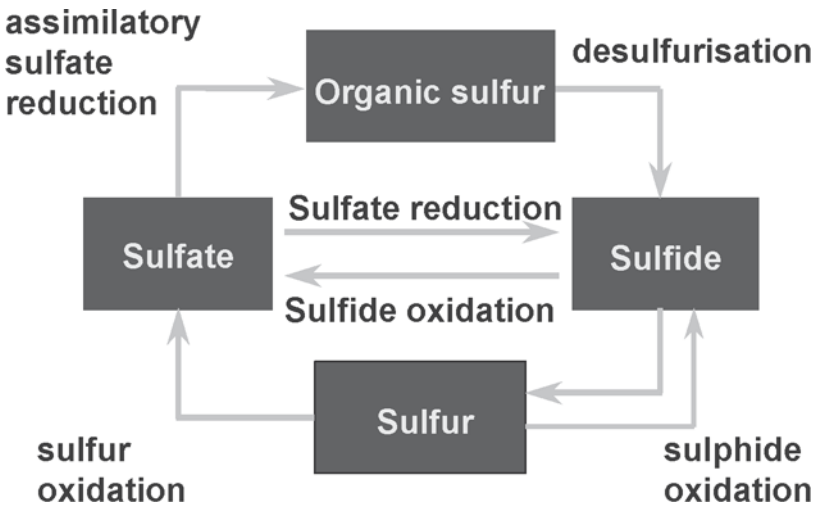

Figure 1. Biological sulphur cycle.

Cork *et al.* (1986) proposed a process, using the green sulphur bacteria *Chlorobium limicola* based on this equation. The major disadvantages in using photosynthetic bacteria on a large scale lie in their anaerobic nature and their requirement for radiant energy and hence extremely transparent solutions. Moreover, many of these organisms accumulate elemental sulphur internally, which would make the separation of sulphur and biomass difficult. Therefore, Kim *et al*. (1993) immobilized cells of *Chlorobium limicola* in strontium alginate beads in order to entrap the formed sulphur. Several researchers described the oxidation of sulphide to elemental sulphur or sulphate using chemolithoautotrophic bacteria belonging to the genus *Thiobacillus* (Beudeker *et al*., 1982; Buisman, 1990; Cadenhead and Sublete, 1990; Cho *et al*., 1992, Gadre, 1989; Ongcharit *et al.*, 1991; Sublette and Gwozdz, 1991). Under sulphide limitation in the reactor, *Thiobacilli* can successfully compete with the chemical oxidation of sulphide because of their high affinity for this compound. Members of the genus *Thiobacillus* are classified as Gram-negative, rod-shaped, colorless sulphur bacteria which utilize reduced inorganic sulphur compounds as their energy source and CO_2 as their main source of carbon. Some species are able to use organic carbon as a supplementary carbon source (Kelly, 1989; Robertson and Kuenen, 1991). Due to their simple nutritional requirements the use of chemolithotrophs for the removal of H_2S is advantageous. Cho *et al*. (1992) extensively reported on H_2S removal by the heterotrophic bacterium *Xanthomonas* sp. strain DY44. The specific H_2S removal rate of this bacterium is however lower than those of purified *Thiobacillus* spp. (Cho *et al*., 1991). Furthermore, application of a heterotrophic organism is not

favorable if organic compounds are not readily available, *e.g.* in gas-purification. Table 3 provides examples of the many types found among the colorless sulphur bacteria, together with some of their environmental requirements.

Table 3.
Sulphur oxidizing bacteria (After: Schlegel, 1992).

Species	pH of growth	Electron donor	Type[a]
Thiobacillus thiooxidans	2-5	S^{2-}, $S_2O_3^{2-}$, S	o
Thiobacillus ferrooxidans	2-6	Fe^{2+}, $S_2O_3^{2-}$, S	f
Thiobacillus thioparus	6-8	CNS^-, $S_2O_3^{2-}$, S	o
Thiobacillus denitrificans	6-8	CNS^-, $S_2O_3^{2-}$, S	o
Thiobacillus intermedius	2-6	$S_2O_3^{2-}$, S, glutamate	f
Thiobacillus novellus	6-8	$S_2O_3^{2-}$, S, glutamate	f
Thiomicrospira pelophila	6-8	S^{2-}, $S_2O_3^{2-}$, S	o
Sulfolubus acidocaldarius	2-3	S, glutamate, peptone	f

[a] o, Obligately autotrophic; f, facultatively autotrophic

According to Kuenen (1982) the following two biological overall reactions may occur in an aerobic sulphide removal system:

$$2\ HS^- + O_2 \rightarrow 2\ S° + 2\ OH^- \qquad \Delta G° = -169.35\ kJ/mol \qquad (1)$$

$$2\ HS^- + 4\ O_2 \rightarrow 2\ SO_4^{2-} + 2\ H^+ \qquad \Delta G° = -732.58\ kJ/mol \qquad (2)$$

Since the formation of sulphate yields most energy, this reaction is preferred by the micro-organisms. The formation of sulphur will only proceed under oxygen-limiting circumstances whereas sulphate is the main product in the presence of an excess amount of oxygen.(Buisman *et al*., 1991; Janssen *et al.*, 1995). High sulphate levels are undesirable as they unbalance the natural sulphur cycle and can cause taste and alimentary problems in drinking water. Since the non-soluble sulphur can be removed, this process enables a reduction of the total sulphur content from the wastewater. Moreover, the sulphur can be re-used as a valuable raw-material in for instance soil-bioleaching processes (Tichy *et al*., 1994) or it can be purified. For this reason, the research carried out at the Sub-Department Environmental Technology at Wageningen University focusses on the development of 'sulphur technologies' that lead to elemental (bio)sulphur as the main end-product. The latest development is the use of halo-alkaliphilic sulphide oxidizing bacteria to limit the water intake. As these micro-organisms thrive at salt concentrations above 2 mol/L less dilution water is needed (Van den Bosch *et al*. 2007; Sorokin *et al*., 2005).

The reductive side of the biological sulphur cycle has been described extensively (Visser *et al*., 1993; Van Houten *et al*., 1994; Weijma *et al*., 2000; Lens *et al*., 2002; Lomans *et al*., 1999). In the presence of a suitable electron donor oxidized sulphur species such as thiosulphate and sulphate are reduced to hydrogen sulphide. Biological sulphide production is also commercially applied to remove metals from polluted groundwater or from aqueous (process) streams in the mine and metal industry by the formation of the corresponding metalsulphide precipitates (Dijkman *et al*., 2002). An example hereof is the sulphate-reducing bioreactor built by Paques B.V. at Pasminco (Budel, The Netherlands). The unit operates with hydrogen gas produced in a steam reformer ($CH_4 + 2H_2O \rightarrow CO_2 + 4H_2$).

3.3 Properties of biologically produced sulphur

In 1887, Winogradsky described the build-up and disappearance of sulphur inclusions by *Beggiatoa*, depending on whether or not the aqueous medium contained H_2S (Trüper and Schlegel, 1964). Many authors have since described the formation and the properties of this «elemental» sulphur for both phototrophic bacteria (Guerrero *et al*., 1984; Hageage *et al*., 1970; Schmidt *et al*., 1971; Steudel *et al*., 1990; Strohl *et al*., 1981; Trüper and Hathaway, 1967) and aerobic *Thiobacilli*. (Jones and Benson, 1965; Steudel *et al.*, 1989; Javor *et al*., 1990; Janssen *et al*., 1994). According to these reports, S° forms transparent droplets (globules) which are deposited inside or outside the cells. The droplets reach diameters of up to 1 mm, are of spherical or ellipsoidal shape and dissolve at least partly in various organic solvents like acetone, chloroform, ethanol and carbon disulfide. Biologically produced sulphur is hydrophilic and has a white or pale-yellow colour. The buoyant density of S° produced by *Chromatium* has been determined at 1.22 $g \cdot cm^{-3}$ (Guerrero *et al*., 1984). When allowed to stand in the liquid state or on drying, the sulphur globules eventually convert to crystalline S_8. However, it has never been demonstrated that the sulphur globules consist of 100 percent sulphur. Surprisingly, many of the properties reported above do not match the properties of any known chemical allotrope of elemental sulphur, indicating that biologically produced sulphur is a not a standard sulphur form. The biologically produced sulphur (or 'biosulfur') is of oxidation state zero and is therefore often described as S^0, although it should not be mistaken for atomic sulphur. In this paper we will use the term biologically produced sulphur to refer to sulphur produced in biotechnological H_2S removal processes. The exact nature of the biologically produced sulphur particles produced in the process is not completely clear but it is likely that sulphur in the particles is present as S_8 rings. Studies by X-ray absorption near edge spectroscopy (XANES) indicated that sulphur globules produced by chemotrophic bacteria, such as the dominating organism *Thiobacillus* sp. W5 (Visser *et al.*, 1997), consist of either S_8 rings or polythionates (Prange *et al.*, 2002). Because polythionates are not stable at the slightly alkaline reactor conditions of the process, their presence

in the sulphur particles is not likely. X-ray diffraction studies showed that sulphur rings are present in the sulfur particles produced in this process and it is therefore most likely that the sulfur atoms are present as S_8 rings (Janssen *et al.*, 1994, 1999). Biologically produced sulphur is hydrophilic and dispersible in water, contrary to 'inorganic' elemental sulfur which is very poorly soluble in water (5 μg L^{-1}) and strongly hydrophobic (Boulègue, 1978). The bio-sulphur particles consist of a core of sulphur, on the surface of which polymeric organic compounds such as proteins are adsorbed. These organic polymers give sterical and electrical stabilization of the colloidal particles. The hydrophilic properties of bio-sulphur become clear from Figure 2.

Figure 2. Hexadecane-water partition-test. Standard hydrophobic yellow 'sulphur flower' remains in the upper hexadecane-phase (left) whereas biologically produced sulphur remains in the lower water phase (right). (Taken from Janssen *et al*., 1999).

3.4 New process for H_2S removal from gas streams

In 1985 laboratory research was initiated at Wageningen University into applications of the biological sulphur cycle to mitigate environmental problems (Rinzema and Lettinga 1988; Buisman *et al*., 1990). After performing extensive pilot plant research Paques B.V. commercialised the first units for biogas desulphurisation and sulphate removal from groundwater. In the mid-nineties, the technology was further developed together with Shell Global Solutions Int. B.V. for a.o. high pressure natural gas and refinery gas desulphurisation.

This new process is based on the bioscrubber principle. First, the sulphur containing compounds are absorbed into the liquid phase whilst in the second process step the microbiological conversion to elemental sulphur and hydroxyl ions takes place, according to the following reaction equation:

$$H_2S + OH^- \rightarrow HS^- + H_2O$$
$$HS^- + 0.5\ O_2 \rightarrow S^\circ + OH^-$$

It can be seen that the hydroxyl ions that are consumed in the first step are regenerated in the second process step.

At excess oxygen conditions a complete oxidation to sulphate takes place, thereby leading to an acidification of the medium:

$$HS^- + 2\ O_2 \rightarrow SO_4^{2-} + H^+$$

For this reason the process should be operated at oxygen-limiting conditions as described earlier (Janssen *et al*., 1995; Kleinjan *et al.,* 2006). In the figure below a schematic representation of this new process for the desulphurisation of a.o. biogas, landfill gas and natural gas is given:

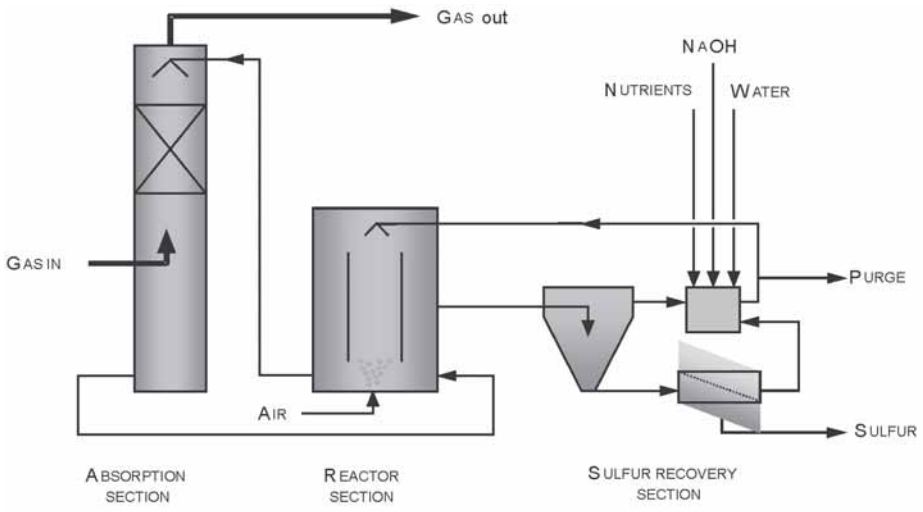

Figure 3. Biotechnological process for gas desulphurisation.

The gas enters a scrubber column and is desulphurized with a slightly alkaline fluid. The cleaned gas leaves the scrubber at the top section. The spent scrubber liquid is collected in the bottom of the scrubber and directed to the bioreactor. Here, air is dispersed at the bottom in order to enable the biomass to convert the dissolved sulphide into elemental bio-sulphur thereby regenerating caustic soda. The sulphur particles are separated by gravity settlement whereafter dewatering takes place, *e.g.* in a decanter centrifuge whilst the clear filtrate is returned to the reactor. The bioreactor effluent is recycled to the scrubber for renewed removal of H_2S. The small sulfate production necessitates a continuous bleed from the unit that is taken from the bioreactor.

The first full scale unit for high pressure natural gas treatment is located in Bantry (Alberta, Canada) near the town of Brooks and is owned and operated by EnCana Resources, a major Canadian and global gas producer. The natural gas is extracted from well sites that are on, or adjacent to the properties of over forty Canadian landowners around the Bantry North facility. The Shell-Paques biological technology was selected because it was the best available technology for this application. The alternative was acid gas re-injection, which was too expensive and therefore not attractive. The Shell-Paques unit is designed to meet a H_2S specification of less than 4 ppm volume on the treated natural gas.

Figure 4. First commercial full-scale unit for biotechnological H_2S removal from high pressure natural gas. The Shell-Paques unit is designed to meet a H_2S specification of <4 ppm volume on the treated natural gas.

The license agreement between EnCana Resources and New Paradigm was signed in the last week of November 2001. Basic engineering started directly in December and detailed engineering was completed by April 2002. Late April the constructor was selected and start was made with the construction of the facility on two skids. These skids were transported to site at the end of July. The construction and the tie-ins to the existing Bantry North infrastructure were done in August. The unit was handed over to the start-up team on 11 September and taken on stream on 12 September 2002. A photograph of the unit is shown below. As the installation is located in a remote area, dedicated tanks for make-up water and bleed water storage were required. Also a caustic storage tank is present and a compost filter for bioreactor vent-air treatment in case H_2S is present in the vent-air during upset conditions.

Monitoring of the unit provided a wealth of information from which we have prepared some graphs to show the most important parameters. The most important observations are shown in Figures 5 and 6.

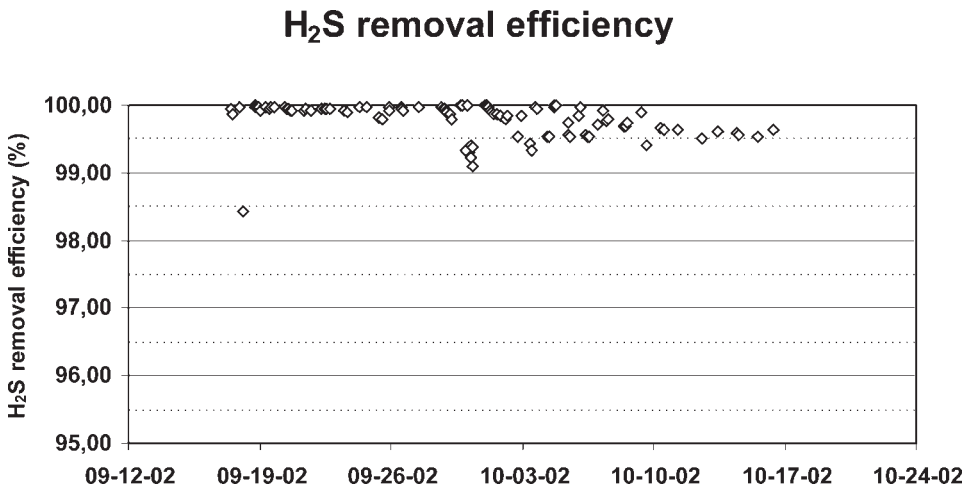

Figure 5. H_2S removal efficiency of first commercial full-scale unit for biotechnological H_2S removal from high pressure natural gas.

From Figure 5, it follows that during a 1 month period the H_2S removal efficiency was always above 99.5%. This makes this process very competitive to conventional physico-chemical processes. During this period the natural gas processing capacity fluctuated (data not shown). Despite these fluctuations, the H_2S content in the treated gas was always less than 4 ppmv (Figure 6).

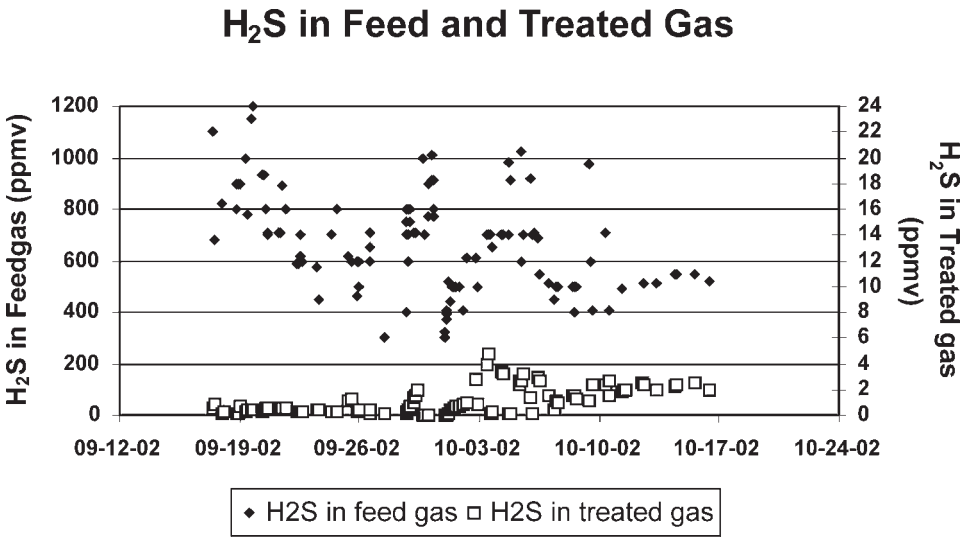

Figure 6. H_2S concentration in untreated (sour) and treated (sweet) gas.

4 CONCLUSIONS

Naturally occurring bacteria of the genus *Thiobacillus* can be used to remove H_2S from gaseous streams whilst producing re-usable elemental sulphur. At present 5 full scale installation are in operation to treat high pressure natural gas and refinery gas. For the treatment of biogas and landfill gas around 80 installations are in operation, worldwide. Important advantages over existing technologies are:

- High removal efficiency for hydrogen sulfide from sour gas.
- High biological activity, so that peak load and other variables in the production processes can be handled effectively.
- Short system start-up time.
- Easily controlled process.
- Operation at ambient temperature.
- Operation at wide pressure range (0 to 80 bar).
- Very low operational costs.
- No sulfide containing waste stream.
- No use of chemical chelating agents.
- No hazardous bleed streams.
- Beneficial use of produced elemental sulfur (agricultural).

References

Aneja, V.P. (1990) Natural sulfur emissions into the atmosphere. *J. Air Poll. Control Assoc.* 40: 469-476.

Beudeker, R.F., Gottschal, J.C. and Kuenen, J.G. (1982) Reactivity versus flexibility in thiobacilli. *Antonie Leeuwenhoek.* 48: 39-51.

Boulègue, J. (1978). Solubility of elemental sulphur in water at 298 °K. *Phosphorus and Sulphur* 5: 127-128.

Brimblecombe, P., Hammer, C., Rohde, H., Ryaboshapko, A. and Boutron, C.F. (1989). Human influence on the sulphur cycle. In: P. Brimblecombe and A. Yu Lein (Eds.) Evolution of the global biogeochemical sulphur cycle. Scope 39, pp 77-121. Wiley, New York.

Buisman, C.J.N., Geraats, B.G., Ijspeert, P. and Lettinga G. (1990) Optimization of sulphur production in a biotechnological sulphide removing reactor. *Biotechnol. Bioeng*. 35: 50-56.

Buisman, C.J.N., Ijspeert, P., Hof, A., Janssen, A.J.H., Ten Hagen, R. and Lettinga G. (1991) Kinetic parameters of a mixed culture oxidizing sulfide and sulfur with oxygen. *Biotechnol. Bioeng.* 38: 813-820.

Cadenhead, P. and Sublette, K.L. (1990) Oxidation of hydrogen sulfide by *Thiobacilli*. *Biotechnol. Bioeng.* 35: 1150-1154.

Cho, K.S., Zang, L., Hirai, M. and Soda M. (1991) Removal characteristics of hydrogen sulfide and methanethiol by *Thiobacillus sp*. isolated from peat in biological deodorization. *J. Ferment. Bioeng.* 71: 44-49.

Cho, K.S., Hirai, M. and Shoda, M. (1992). Enhanced removal efficiency of malodorous gases in a pilot-scale peat biofilter inoculated with *Thiobacillus thioparus DW44*. *J. Ferment. Bioeng.* 71: 46-50.

Cork, D.J., Jerger, D.E. and Maka, A. (1986) A biocatalytic production of sulfur from process waste streams. *Biotechnol. Bioeng. Symp. Ser.* 16: 149-162.

Dignon, J. and Hameed, S. (1989) Global emissions of nitrogen and sulphur oxides. *J. Air Poll. Control Assoc.* 39: 180-186.

Dijkman, H., Boonstra, J., Lawrence, R. and Buisman, C.J.N. (2002) Optimization of metallurgical processes using high rate biotechnology. Paper presented at the TMS 2002 annual meeting, Seattle, USA.

Earthwatch United Nations Environment Program (1992) Chemical Pollution: A global Overview. The International Register of Potentially Toxic Chemicals and the Global Environment Monitoring System's Monitoring and Assessment Research Centre, Geneva.

Gadre, R.V. (1989) Removal of hydrogen sulfide from biogas by chemoautotrophic fixed-film bioreactor. *Biotechnol. Bioeng.* 34: 410-414.

Groenestijn van, J.W. (2005) Biotechniques for air pollution control: past, present and future trends. Proceedings of the International Congress Biotechniques for Air Pollution Control. La Coruña, Spain, 5-7 October 2005, pp. 3-12.

Guerrero, R., Mas, J. and Pedros-Alio C. (1984) Buoyant density changes due to intracellular content of sulfur in *Chromatium warmingii* and *Chromatium vinosum*. *Arch. Microbiol.* 137: 350-356.

Hageage, G.J. Jr., Eanes, E.D. and Gherna, R.L. (1970) X-Ray diffraction studies of the sulfur globules accumulated by *Chromatium* species. *J. Bacteriol*. 101: 464-469.

Houten Van, R.T., Hulshoff Pol, L.W. and Lettinga, G. (1994) Biological sulphate reduction using gas-lift reactors fed with hydrogen and carbon dioxide as energy and carbon source. *Biotechnol. Bioeng.* 44(5): 586-594.

Janssen, A.J.H., De Keizer, A. and Lettinga, G. (1994) Colloidal properties of a microbiologically produced sulphur suspension in comparison to a LaMer sulphur sol. Colloids Surfaces B: *Biointerfaces* 3: 111-117.

Janssen, A.J.H., Sleyster, R., Van der Kaa, C., Jochemsen, A., Bontsema, J. and Lettinga, G. (1995) Biological sulphide oxidation in a fed-batch reactor. *Biotechnol. Bioeng.* 47: 327-333.

Janssen, A.J.H., Lettinga, G. and de Keizer, A. (1999) Removal of hydrogen sulphide from wastewater and waste gases by biological conversion to elemental sulphur. Colloidal and interfacial aspects of biologically produced sulphur particles. *Colloid. Surf. A-Physicochem. Eng. Asp.*, *151*, 389.

Janssen, A.J.H., Buisman, C.J., Kijlstra, W.S. and Grinsven, P.F.A. van (1999) The Shell-Paques desulfurisation process for H2S removal from high pressure natural gas, synthesis gas and Claus tail gas. In: Proceedings Ninth Gas Research Institute Sulfur Recovery Conference, October 24-27, 1999, San Antonio, Texas.

Javor, B.J., Wilmot, D.B. and Vetter, R.D. (1990) pH-Dependent metabolism of thiosulfate and sulfur globules in the chemolithotrophic marine bacterium *Thiomcrospira crunogena*. *Arch. Microbiol.* 154: 231-238.

Jensen, A.B. and Webb, C. (1995). Treatment of H_2S-containing gases: A review of microbiological alternatives. *Enzyme Microb. Technol.* 17: 2-10.

Jones, G.E. and Benson, A.A. (1965) Phosphatidyl glycerol in *Thiobacillus thiooxidans*. *J. Bacteriol.* 89: 260-261.

Kelly, D.P. (1989) Physiology and biochemistry of unicellular sulfur bacteria, In: Autotrophic Bacteria. H.G. Schlegel and B. Bowien (Ed.), 193-213. Springer-Verlag, Berlin.

Kleinjan, W.E., De Keizer, A. and Janssen, A.J.H. (2003) Biologically produced sulfur. *Top. Curr. Chem.* 230: 167-188.

Kleinjan, W.E., Lammers, J.N.J.E., De Keizer, A. and Janssen A.J.H. (2006) Effect of biologically produced sulfur on gas absorption in a biotechnological hydrogen sulfide removal process. *Biotechnol. Bioeng.* 94(4): 633-644.

Kuenen, J.G. (1975) Colourless sulphur bacteria and their role in the sulphur cycle. *Plant Soil* 43: 49-76.

Lens, P., Vallero, M., Esposito, G. and Zandvoort, M. (2002) Perspectives of sulfate reducing bioreactors in environmental biotechnology. *Reviews in Environmental Science and Bio/ Technol.* 1(4): 311-325.

Lide, D.R. (Ed.) (1995) Handbook of Chemistry and Physics, 75th edition, CRC Press Boca Raton, M.A.

Lomans B.P., Camp, H.J.M. op den, Pol, A. and Vogels, G.D. (1999) Anaerobic versus aerobic degradation of dimethyl sulfide and methanethiol in anoxic freshwater sediments. *Appl. Environ. Microbiol.* 65: 438-443.

Niel van, C.B. (1932) On the morphology and physiology of the purple and green sulphur bacteria. *Arch. Microbiol.* 3: 1-112.

O'Brien, M., Wentworth, C., Lanning, A. and Engert, T. (2007) Shell-Paques® Bio-Desulfurization process directly and selectiveley removes H_2S from high pressure natural gas – start up report. In: Proceedings of the 57th Annual Laurance Reid Gas Conditioning Conference, University of Oklahoma, Norman, Oklahoma.

Ongcharit, C., Sublette, K.L. and Shah, Y.T. (1991) Oxidation of hydrogen sulfide by flocculated *Thiobacillus denitrificans* in a continuous culture. *Biotechnol. Bioeng.* 37: 497-504.

Prange, A., Chauvistre, R., Modrow, H., Hormes, J., Trüper, H.G. and Dahl, C. (2002) Quantitative speciation of sulfur in bacterial sulfur globules: X-ray absorption spectroscopy reveals at least three different species of sulfur. *Microbiology.* 148: 267.

Rinzema, A. and Lettinga, G. (1988) Anaerobic treatment of sulphate containing waste water, pp. 65-109. In D.L. Wise (Ed.)., Biotreatment systems, 3. CRC Press, Boca Raton, Fl.

Robertson, L.A. and Kuenen, J.G. (1991) The colorless sulfur bacteria, 385-413. In: A. Balows, H. Trüper, M. Dworkin, W. Harder, K.-H. Schleifer (Eds.), The prokaryotes, 2nd edition, Springer-Verlag, New York.

Schlegel, H.G. (1992), General Microbiology, 7th ed., Cambridge University Press, U.K.

Schmidt, G.L., Nicolson, G.L. and Kamen, M.D. (1971) Composition of the sulfur particle of *Chromatium vinosum* strain D. *J. Bacteriol.* 105: 1137-1141.

Sorokin, D.Y., Banciu, H., van Loosdrecht, M. C. M. and Kuenen, J.G. (2003) Growth physiology and competitive interaction of obligately chemolithoautotrophic, haloalkaliphilic, sulfur-oxidizing bacteria from soda lakes. *Extremophiles.* 7: 195-203.

Steudel, R. (1989). On the nature of the «Elemental Sulfur» (S°) produced by sulfur oxidizing bacteria- a model for S° globules. in: Autotrophic Bacteria. H.G. Schlegel and B. Bowien (Eds.), Science Tech Publishers, Madosin, WI.

Steudel, R., Holdt, G., Visscher, P.T. and Van Gemerden, H. (1990) Search for polythionates in cultures of *Chromatium vinosum* after sulfide incubation. *Arch. Microbiol.* 153: 432-437.

Steudel, R., Göbel, T. and Holdt, G. (1988) The molecular composition of hydrophilic sulfur sols prepared by acid decomposition of thiosulfate. *Z. Naturforsch.* 43b: 203-218.

Strohl, W.R., Geffers, I. and Larkin, J.M. (1981) Structure of the sulfur inclusion envelopes from four Beggiatoas. *Current Microbiology.* 6: 75-79.

Sublette, K.L. and Gwozdz, K.J. (1991) An economic analysis of microbial reduction of sulfur dioxide as a means of byproduct recovery from regenerable processes for flue gas desulfurization. *Appl. Biochem. Biotech.* 28/29: 635-646.

Tichý, R., Janssen, A., Grotenhuis, J.T.C., Lettinga, G. and Rulkens, W. (1994) Possibilities for using biologically-produced sulphur for cultivation of *Thiobacilli* with respect to bioleaching processes. *Biores. Technol.* 48: 221-227.

Trüper, H.G. and Schlegel, H.G. (1964) Sulphur metabolism in Thiorhodaceae. I. Quantitative measurements on growing cells of *Chromatium okenii*. *Antonie Leeuwenhoek.* 30: 225-238.

Trüper, H.G. and Hathaway, J.C. (1967) Orthorhombic sulphur formed by photosynthetic sulphur bacteria. *Nature.* 215: 435-436.

UNEP (1991) United Nations Environment. Programme Environmental Data Report, Third Edition 1991/92, Basil Blackwell, Oxford.

Van den Bosch, P.L.F., Van Beusekom, O.C., Buisman, C.J.N. and Janssen, A.J.H. (2007) Sulfide oxidation under halo-alkaline conditions in a fed-batch bioreactor. *Biotechnol. Bioeng.* (*accepted for publication*).

Visser, A. Gao, Y. and Lettinga, G. (1993) Effects of pH on methanogenesis and sulphate reduction in thermophilic (55 C) UASB reactors. *Biores. Technol.* 44: 113-121.

Visser, J.M., Stefess, G.C., Robertson, L.A. and Kuenen, J.G. (1997) *Thiobacillus* sp. W5, the dominant autotroph oxidizing sulfide to sulfur in a reactor for aerobic treatment of sulfidic wastes. *Antonie van Leeuwenhoek*. 72: 127.

Weijma, J., Haerkens, J.-P., Stams, A.J., Hulshoff Pol, L.W. and Lettinga, G. (2000) Thermophilic sulfate and sulfite reduction with methanol in a high rate anaerobic reactor. *Water Sci. Technol.* 42(5-6): 251-258.