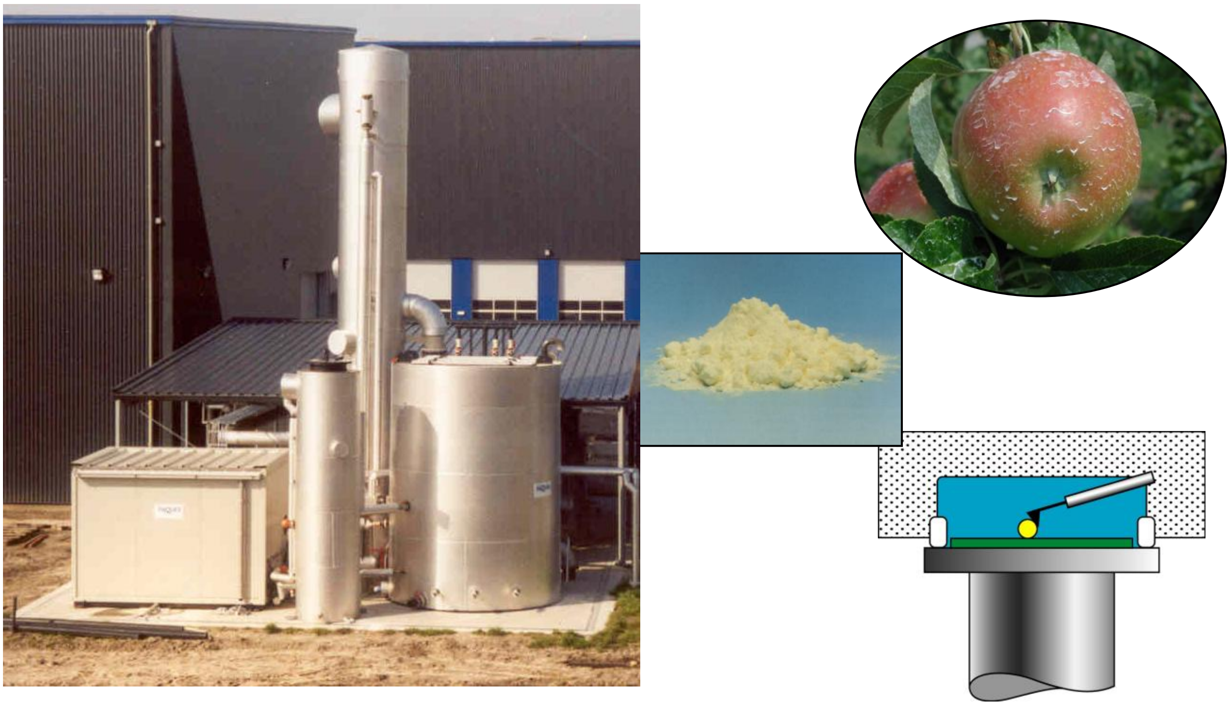
Closing the sulphur cycle

Applications of bio-sulphur



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SUMMARY

Paques BV has developed a technology for biological removal of H_2S from natural and biogas. This technology can also be applied to desulphurize any kind of sulphide-polluted gas stream. The sulphide-rich gas is scrubbed with a slight alkaline solution. In a bioreactor the sulphide is converted to elemental sulphur; Thiopaq™ biosulphur. In general the particle size of biosulphur is small, less than 50 μm. A large difference between biosulphur and Claus sulphur is the hydrophilic nature of biosulphur. The small particle size and hydrofilicity of the biosulphur makes application in agricultural attractive. In this study the application of Thiopaq™ biosulphur as fertilizer and as fungicide was studied. The objective of this study was the come forward with new methods for reusing Thiopaq™ biosulphur in a sustainable way, without landfilling the sulphur as chemical waste.

In the bioreactor the sulphur is present as a diluted sludge. This sludge is concentrated with a gravity settler and a centrifuge up to a thick paste (~60w%). The resulting paste was dried to a powder of 90w%. Different types of dryers were used and evaluated. The powders produced were physical-chemical characterized. A market study for both biosulphur as fungicide and as fertilizer was performed. The efficacy of the Thiopaq™ biosulphur as fungicide was compared with a commercial product (Thiovit).

Three different dryers were selected based on the particle properties (melting temperature, crystallinity), product specifications (particle size, and water content) and feed properties (flow behavior and water content): a high speed paddle dryer, a spray dryer, and a inert medium spouted bed dryer. For all three dryers pilot plant test runs were performed, and it was concluded that acceptable operation was obtained with a paddle dryer and an inert medium spouted bed dryer. As the spouted bed dryer produces a powder with a smaller particle size than the paddle dryer, the spouted bed dryer is the preferred choice.

The dried biosulphur particles were characterized for surface and colloid-chemical properties and the interaction of biosulphur particles with model and leaf surfaces were determined. The characterization focused on 1) particle size and particle size distribution, on 2) wetting behavior of the particles and 3) on the attachment force of the particles to different surfaces. In comparing the native biosulphur with dried biosulphur differences were noted in 1) specific surface area, 2) contact angles, and 3) methylene blue adsorption. Biosulphur particles are hydrophilic due to an organic polymeric layer attached to the particles surface. It seems that this polymeric layer was changed due to drying. The wetting behavior was different between native and dried biosulphur. However, the surface charge as obtained from microelectroforesis is similar for the dried and native samples. It appeared that the biosulphur has lost part of its hydrophilicity, but the biosulphur is still more hydrophilic than Claus sulphur.

The particles size distribution depended to a small extent on the type of dryer used. The flash dryer gave a single size fraction, whereas for the other dryers fractions with a diameter ranging from 16 to 200 μm were observed. In all cases aggregates were detected. By sonification these aggregates could be destroyed and primary particles with a diameter of 2 μm were obtained. Images of biosulphur particles were obtained with atomic force microscopy (AFM). These images clearly showed the crystalline character of the biosulphur particles.

AFM was used to measure the interaction between sulphur particles and different surfaces. Results showed that the formulated Thiovit particle (a commercial sulphur-based fungicide) gave an attractive contact force, whereas a small repulsion was observed for the unformulated Thiopaq particles. In this study it was clearly demonstrated that AFM could be used to evaluate the influence of additives on the interaction between particle and surface.

Sulphur is the most important fungicide used in organic production. In organic apple production it is the only fungicide effective against the main disease, apple scab, caused by *Venturia inaequalis*. The efficacy of Thiopaq™ biosulphur as a fungicide was tested in three types of experiments: 1) germination test of conidiospores of *Venturia inaequalis*, 2) an efficacy test against mildew on cucumber and 3) a rain fastness test with apple scab. The efficacy was compared with the commercial sulphur-based fungicide Thiovit Jet .The results of these experiments showed that Thiopaq™ biosulphur was more effective than Thiovit Jet in the control of apple scab and mildew. In a rainfastness test potted apple trees were treated with the sulphur. Next, they were subjected to a known amount of artificial rain. Then the apple trees were inoculated with apple scab and placed in climate chamber. From this test it was concluded that the efficacy of Thiopaq™ sulphur was not significantly different from that of Thiovit Jet for scab incidence, when the severity is expressed as the number of leaves infected. However, looking at scab severity expressed as the number of lesions (spots) of scab per pot than, Thiopaq sulphur was significantly more effective than Thiovit Jet. This result was similar to the results on the efficacy against mildew or cucumber.

Previous research showed that Thiopaq™ biosulphur applied as fertilizer gave an increase in crop yield of canola of 10% compared with commercial sulphur-based fertilizers. So, Thiopaq™ biosulphur appears to be attractive for application in agriculture as a fungicide and fertilizer.

A cost prize calculation was performed for two different types of areas were the biological desulphurization technology is applied: biosulphur produced from biogas and biosulphur produced from natural gas. The difference between both applications is the size of the production plant; a biogas plant typically produces 100 – 400 kg sulphur/day, and a natural gas plant several tons sulphur/day. For a biogas plant, sulphur sludge is concentrated with only a gravity settler, whereas for a natural gas plant a centrifuge is part of the initial investment. Cost prize calculations showed that for the biogas application the most economical viable option is collecting the biosulphur on site, concentrating the biosulphur sludge with a mobile centrifuge, and sending the paste to a central point. The total costs will be between € 80 to 167 /ton S. When the paste is further dried at the central point to a powder the additional cost depend on the scale. Based on the scale this adds € 670 ton/S for a capacity of 1 ton/day and € 430 ton/S for a capacity of 3 ton/day. The preferred form from a cost prize viewpoint is concentrated slurry. For the natural gas application production volumes are larger, and a centrifuge is part of the initial investment. The additional costs are only the drier investment (€290 /ton S for 3 ton S/day, and € 27/ ton S for 14 ton S/day).

For biosulphur both as fertilizer and as fungicide a market study was performed. For biosulphur as fertilizer it is concluded that there is large market potential as there is an increasing interest for sulphur-based fertilizers. However, the current amount of produced bio-sulphur is not enough to play an important role as sulphur fertilizer. For the moment the combination of locale production and locale sulphur-fertilizer demand is the only economically feasible way of using bio-sulphur as fertilizer. In order to gain a significant market share, significant amounts of bio- sulphur will have to be produced.

The world annual market for biosulphur as fungicide is between 180 and 200 Meuro, and the total volume is about 200,000 tonnes. Application in fruit protection is about 170.000 tons/year, of which grape is the major consumer (120.000 ton/year).The amounts used depend on the crop to be protected, and vary between 1 kg/ha and 30 kg/ha. Grape uses most sulphur per hectare (almost 16 kg per hectare). It is anticipated that the demand for sulphur as crop protecting agent will grow based on 1) a growing acreage and production in the developing countries, 2) a tendency to a more severe regulation in the EU towards crop protecting agents and 3) a growing acreage of fruit in general and grape in particular.

Results showed in this study demonstrate a great potential for Thiopaq™ biosulphur in agricultural applications. For further development the route is different for both applications.

For Thiopaq™ biosulphur applied as fungicide different companies were approached. These companies see biosulphur very suitable for the application in the sustainable agriculture market. However, before Thiopaq™ biosulfur can be applied as fungicide, it needs to be approved by the Dutch Government. This is a lengthy and an expensive process. This lengthy process creates uncertainties, such as will the market still exist, and will the product be approved. An alternative would be to market the new product under the wings of a company that already has sulphur approved as a fungicide component. The search for a partner needs to be concluded and the Thiopaq™ biosulphur needs to be further developed into an attractive active component of a commercial fungicide.

For application as fertilizer, it should be relatively easy to get approval. After approval, a series of field tests can be organized for marketing purposes. The next season the sulfur could be sold commercially. Two companies were approached. Both companies named the low prices for sulphur based fertilizers as the biggest hurdle for success. As the fact the product is biologically produced and its particle size is small, a good potential is seen in certain niche markets like biological farming. A significant market for bio-sulfur as fertilizer is likely to be created after investigation of possible partnerships with fertilizer producers on one side and potential Paques clients for Thiopaq technology on the other side.

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1. INTRODUCTION

Paques BV has developed a biological process for the desulphurisation of biogas; the Thiopaq™ process. The first plant was put into operation in 1993 at Industriewater Eerbeek. Nowadays over 20 plants are worldwide in operation. Countries in which Thiopaq installations have been sold are The Netherlands, Germany, Denmark, Spain, Italy, Austria, Czech republic, Egypt, India, Chili, US. Together with Shell Global Solutions International the Thiopaq™ process was further developed for desulphurisation of natural gas (called Shell-Paques process). The Thiopaq™ process principle can be used for the removal of H_2S from gases like biogas, natural gas, gasification gases, process gases and (ventilation) air. Thus far, the process has successfully been applied for biogas, refinery sour gas, natural gas and ventilation air.

With the Thiopaq™ process H_2S is converted biologically to elemental sulphur.
The most important features of the process are: 1) Very high removal efficiencies for hydrogen sulphide from process gases; the dangerous H_2S is absorbed in a mild alkaline solution, 2) no need for use of chelating compounds (chemical RedOx processes) and no subsequent production of hazardous bleed streams. 3) very low operational costs due to recovery of caustic soda (>93% savings on caustic soda use compared to caustic scrubbers for oxygen free gas streams), 4) production of elemental sulphur as re-usable byproduct.

Up to now biosulphur is discarded as chemical waste, or completely oxidized in an aerobic wastewater treatment plant. However, a better option for discarding the biosulphur would be to reuse it in agriculture as fertilizer or fungicide. It is known that due to the reduced emission of SO_2 by adoption of pollution control measures in industrial countries, there is a widespread S deficiency in several highly S-demanding crops (e.g. oilseed rape and cereals) in European countries. In the world single superphosphate (12% S) and ammoniumphosphate (2% S) are the most common forms of sulphur supply, followed by compound fertilizers. One of the problems of sulphate fertilizers is the high leachability of sulphate ions. Therefore the fertilizer industries are promoting elemental sulphur as the most concentrated sulphur fertilizer source with high antifungal properties (Ernst, 2000).

Standard *hydrophobic* sulphur has to be covered with a biosurfactant before it can be oxidized to sulphate. Sulpher is a slow release fertilizer, which cannot be taken up by the plants, but has to be oxidized to sulphate. Biosulphur particles, however, are covered with a thin polymer layer, which make these particles *hydrophilic*. Consequently, biosulphur particles can be easier oxidized. Moreover, these biosulphur particles are rather small, as bacteria produce them, and thus there specific area is large. Based on these features, it is expected that biosulphur is a good starting material as fertilizer or as fungicide.

1.1. Process background

The process of removing H_2S from gas consists of three sections. These include an absorber, an aerobic (biological) reactor and a sulphur separation step. A schematic overview of the system is shown in figure 1.1.

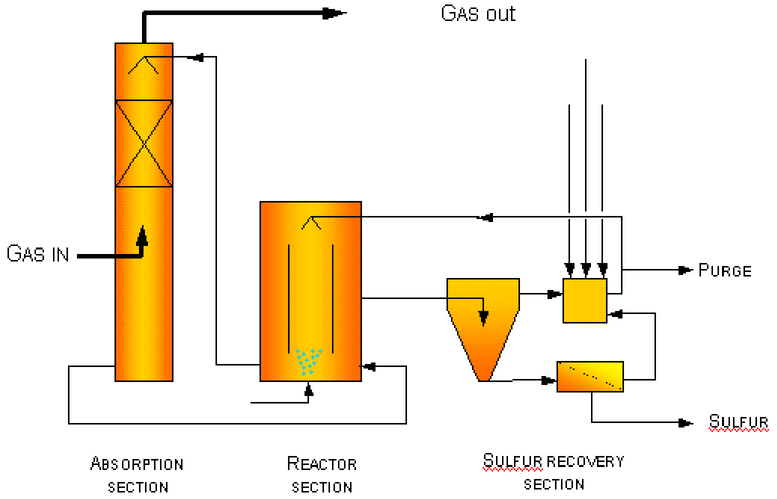

Figure 1.1. THIOPAQ® process for gas desulphurization

The gases enter a wet scrubber, typically a packed column, and are desulphurized with a slightly alkaline fluid. The cleaned gases leave the scrubber at the top.

The spent scrubber liquid is collected in the bottom of the scrubber and directed to the bioreactor. In the reactor air is dispersed at the bottom in order to enable the biomass to convert the dissolved sulphide into elemental sulphur, thereby regenerating caustic soda. We will refer to this biological produced sulphur (short: biosulphur) in a bioreactor developed by Paques as “Thiopaq™ sulphur”.

The sulphur is separated as a solid and the sulphur slurry can optionally be pumped into a sulphur recovery unit. In this unit the slurry can be dewatered and discharged as a cake for further use. The liquor is returned to the reactor.

The bioreactor effluent is recycled to the scrubber for renewed removal of H_2S. From the system a small bleed stream is taken in order to prevent any built up of formed salts.

Process Chemistry

In a scrubber hydrogen sulphide is absorbed under alkaline conditions, i.e. at pH 8 - 9. The absorption of H_2S proceeds according to the following equation:

$$H_2S + NaOH \rightarrow NaHS + H_2O$$

From this equation it follows that alkalinity is consumed.

High H_2S removal efficiency is feasible, because the H_2S concentration in the washing liquid entering the scrubber will be virtually zero.

In the THIOPAQ® process, the alkalinity consumption due to the absorption of H_2S is compensated by the oxidation of hydrogen sulphide to elemental sulphur which proceeds under oxygen controlled conditions:

$$NaHS + ½O_2 \rightarrow S^o + NaOH$$

The THIOPAQ® process uses bacteria of the genera *Thiobacillus* to oxidize the hydrogen sulphide. These bacteria grow very fast and are known to be highly resistant to varying process conditions.

A small part of the dissolved sulphide will be oxidized to sulphate according to:

$$2NaHS + 4O_2 \rightarrow 2NaHSO_4 \leftrightarrow Na_2SO_4 + H_2SO_4$$

As a result of this side reaction, caustic soda is required to neutralize the formed sulphuric acid. A small bleed stream is withdrawn from the system to ensure that the build-up of sodium sulphate and other salts is prevented. The bleed stream (containing sodium salts and some sulphur particles) is harmless and can generally be discharged without problems.

1.2. Background Microbiology

Biologically produced sulphur or biosulphur is a form of sulphur of oxidation state zero that is produced by microorganisms. Kleinjan, de Keizer and Janssen have been published a recent review on the properties of biologically produced sulphur (Kleinjan, De Keizer et al. 2003). This section is mainly based on this review. For detailed references we refer to this review.

A large variety of bacteria that are capable of sulphur formation. The sulphur formed by these bacteria is an intermediate in the oxidation of sulphide or thiosulphate to sulphate and the bacteria are sulphur compound oxidizing bacteria, often called sulphur bacteria (SB). In figure 1.2 the role of these sulphur compound oxidizing bacteria in the biological sulphur cycle is pointed out.

The biological sulphur cycle consists of a continuous oxidation and reduction of sulphur compounds by microorganisms or plants. On the left hand side of the cycle in figure 1.2 the most reduced sulphur compound is shown (sulphide) and on the right hand side the most oxidized form of sulphur (sulphate).

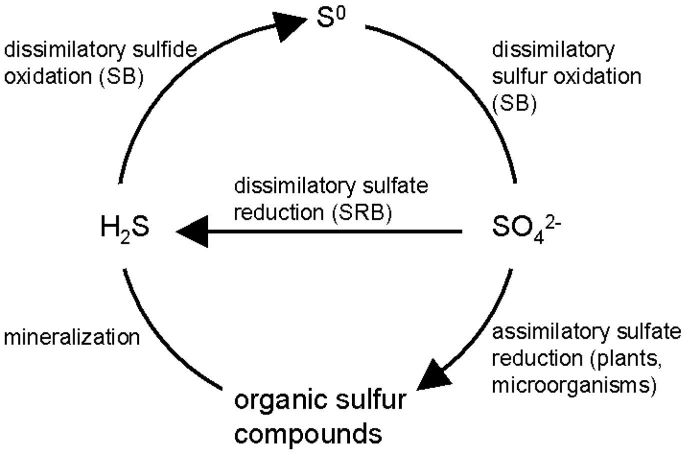

Figure 1.2. The biological sulphur cycle. SB: sulphur compound oxidizing bacteria; SRB: sulphate reducing bacteria.

Sulphur compound oxidizing bacteria cover the oxidation of sulphide to sulphate with reduced sulphur compounds such as sulphur, thiosulphate, and tetrathionate as intermediates. It should be noted that there is a large variety in sulphur compound oxidizing bacteria of which some species are only capable of covering a specific step in the oxidation of sulphide to sulphate (e.g. oxidation of thiosulphate to sulphate). Also the pathways in which the oxidation takes place may vary so that not always the same intermediates are formed. The reduction of sulphate back to sulphide takes place in two ways. In the first way, sulphate reducing bacteria (SRB) reduce sulphate via dissimilatory reduction (inorganic compounds reduced to other inorganic compounds). Secondly, inorganic sulphur compounds can be incorporated into organic substrates, such as proteins, by plants, or other microorganisms (assimilatory reduction). Sulphide is then formed by mineralization of the organic matter.

Sulphur compound oxidizing bacteria. There is a wide variety of sulphur compound oxidizing bacteria consisting of different groups and genera. Each of the bacteria has its own specific properties, such as the source for energy, carbon or hydrogen, the sulphide oxidizing pathway, the size and shape of the bacteria and location of storing intermediate sulphur. In Table 1.1 a number of sulphur compound oxidizing bacteria are listed with some of their distinctive properties. Classification of sulphur compound oxidizing bacteria is however not straightforward. Different bacteria of the same bacterial family can have different properties. Each family consists of a wide variety of genera and strains, which can differ substantially in sulphur-oxidizing capacities or other properties. Therefore, the data in Table 1.1 should be seen only as a list of properties of some sulphur compound oxidizing bacteria. Most important in the classification of sulphur bacteria is the distinction between phototrophic and chemotrophic sulphur bacteria. Phototrophic (purple or green) bacteria use light as energy source to reduce CO_2 to carbohydrates. For biologically production of Thiopaq™ sulphur chemotrophic (colorless) sulphur bacteria obtain energy from the chemical aerobic oxidation of reduced sulphur compounds.

Table 1.1. Properties of some sulphur compound oxidizing bacteria.

Organism	Energy	Carbon source	Sulphur globules	pH of growth
Chlorobiaceae	phototrophic	autotrophic	extracellular	
β-Proteobacteria				
Thiobacillus thioparus	chemotrophic	autotrophic	extracellular	6-8
Thiobacillus denitrificans	chemotrophic	autotrophic	extracellular	6-8
Thiobacillus sp. W5	chemotrophic	autotrophic	extracellular	7-9
γ-Proteobacteria				
Allochromatium vinosum	phototrophic	mixotrophic	intracellular	7.5
Halorhodospira abdelmalekii	phototrophic	mixotrophic	extracellular	8.4
Beggiatoa alba	chemotrophic	mixotrophic	intracellular	
Acidithiobacillus thiooxidans	chemotrophic	autotrophic	extracellular	2-5
Acidithiobacillus ferrooxidans	chemotrophic	mixotrophic	extracellular	2-6
Thioalkalivibrio denitrificans	chemotrophic	autotrophic	extracellular	7.5-10.5
Thioalkalimicrobium cyclicum	chemotrophic	autotrophic	extracellular	7.5-10.5
Xanthomonas	chemotrophic	heterotrophic		7
Cyanobacteria				
Oscillatoria limnetica	phototrophic	autotrophic	extracellular	6.8

With respect to the carbon source, the sulphur compound oxidizing bacteria can be classified as heterotrophs, autotrophs, or mixotrophs. Autotrophic bacteria use CO_2 as the major carbon source for production of organic molecules. Heterotrophic bacteria use organic material as carbon source. Obligate autotrophs strictly need CO_2 as carbon source and facultative autotrophs (or mixotrophs) can also grow heterotrophically. Heterotrophic sulphur compound oxidizing microorganisms need a nutrient feed containing organic substrates (e.g. meat or yeast extracts). Sulphur compound oxidizing bacteria can also be classified according to the nature of the hydrogen source. Lithotrophic bacteria use inorganic substrates (e.g. NH_3, H_2S) as a source for hydrogen atoms. Organotrophic bacteria obtain hydrogen from organic molecules. In most organisms lithotrophy is linked to autotrophy, which means that for instance obligate autotrophic *Thiobacillus* bacteria are also called chemolithotrophic.

Elemental sulphur is often observed as an intermediate product in the oxidation of sulphide to sulphate in sulphur compound oxidizing bacteria. It can be present in considerable concentrations but will eventually be further oxidized to sulphate. The elemental sulphur is stored in sulphur globules, which some bacteria deposit inside the cell membrane and others outside the cell membrane. Below the properties of these sulphur globules are discussed.

The optimum pH of growth of the bacteria differs per species. Neutral and acidiphilic sulphur compound oxidizing bacteria have been long known, mostly isolated from marine or freshwater sediments. From highly alkaline salt lakes a number of alkaliphilic sulphur compound oxidizing bacteria have been isolated, both phototrophic and chemotrophic.

1.3. Applications of biologically produced sulphur

Sulphur produced by microorganisms in H_2S removal plants such as described in the previous section, can be handled in a number of ways. Dried sulphur solids can be used in sulphuric acid production (99% sulphur purity needed) or the formed sulphur sludge can be directed to a smelter where it is converted into high purity sulphur (>99.9%). Unfortunately, currently more sulphur is produced worldwide than is needed as pure chemical and therefore sulphur is also stored in landfill (95-98% sulphur purity needed). Although solid

sulphur is considered as a non-hazardous refinery waste, landfill is an undesirable option, partly because acidification by oxidation has to be prevented.
To avoid the landfill, possible applications of the biologically produced sulphur have been investigated. In bioleaching and in agriculture, it was found that the specific properties of biologically produced sulphur (small particle size, hydrophilic surface) have clear advantages over the use of inorganic sulphur.

Bioleaching is used in mining to dissolve metals from sulphide-ores. The aim of bioleaching is to achieve pH values that are low enough to solubilize a maximum of metals. In some cases additional elemental sulphur is added as substrate for bacteria (Tichý 2000), which oxidize the sulphur forming sulphuric acid, and thereby decrease the pH. Tichý compared the use of sulphur flower with biologically produced sulphur, produced by sulphide oxidation of *Thiobacilli* under oxygen limitation (Tichý, Janssen et al. 1994). He concluded that the hydrophilic properties of biologically produced sulphur have a positive effect in bioleaching because they cause an increase of the rate of sulphuric acid production.

Another field in which biologically produced sulphur can be used is in agriculture. Sulphur is an important nutrient for plants, which can take up sulphur by their leaves from the atmosphere as very reduced (COS, CS_2 and H_2S) up to highly oxidized compounds (SO_2). Most of the sulphur, however, is taken up by plant roots as water-soluble sulphate. Partly due to the decrease in SO_2 emissions since the 1970s, there is a widespread sulphur deficiency in soils that are used for cultivation of several highly S-demanding crops, especially oilseed rape and cereals in Denmark, England, F.R.G. and Scotland. In those circumstances additional S feeding is required. First results of studies (Kijlstra, Janssen et al. 2001) on the yield of canola showed that the grain yield is higher when biologically produced sulphur is used than when other commercially available sulphur sources were used.

Table 1.2. Grain yield of Breton Canola for different sulphur sources.

Sulphur source	Grain yield (kg/ha)
None	14.6
K_2SO_4	15.9
Claus sulphur	17.1
Biosulphur paste	22.3
Biosulphur powder	19.8

1.2 Objective

The objective of this project is to reach new methods for reusing biosulphur in a responsible manner without landfilling this biosulphur as chemical waste. For that, the reuse of biosulphur as fertilizer and as fungicide will be studied. Therefore sulphur powders will be prepared using different dryers. These powders will be physical-chemical characterized. Further the biosulphur will be tested as fungicide and compared with a commercial product. For evaluating the economic perspective of biosulphur both a market study for biosulphur as fertilizer or fungicide will be performed.

2. SULPHUR RECOVERY AND SULPHUR DRYING

In this chapter a process scheme for the recovery of biosulphur from the bioreactor will be described. The recovery consists of concentration the biosulphur and subsequently drying the sulphur.

2.1. Sulphur concentrating

Biosulphur particles are produced in the bioreactor. At normal operation the concentration of biosulphur particles in this bioreactor is between 5 and 15 g/l, which depends on production site-specific conditions. The bioreactor content is sent to a gravitational settler for pre-concentrating this stream. Using a settler the bioreactor content is concentrated up to 70 – 120 g/l. Sizing of the gravitational settler depends on the settling velocity of the sulphur particles. The most important factors are the size, density, and number of particles present, and beneath that pH and conductivity of the sulphur sludge. Typical settling velocities are between 1 - 10 m/h. Any type of sedimentation device can be used, e.g. a funnel type apparatus without any internals, or a tilted plate system. A tilted plate system is funnel with a number of settling plates inside. With this latter apparatus the plot size is largely decreased due to the enhanced settling area. At current production plants this tilted plate system function satisfactory. The clarified solution is send back to the bioreactor.

Further concentrating the biosulphur slurry is achieved with a decanter-centrifuge. Commercially available centrifuges are able to concentrate the slurry up to 450 – 600 g/l. There is good in field experience with the product of Flottwegg. The decanter-centrifuge of Pieralis is also capable of concentration the sulphur slurry up to 600 g/l. The clarified solution is sent back to the bioreactor.

This slurry paste is collected in a conical mixer. The slurry is kept in motion with a screw mixer. Good in field experience exist with a conical mixer from Vrieco-Nauta. This paste is pumped to the dryer for producing a dry powder. Different types of dryers were tested to produce the sulphur powder.

2.2. Drying

There are an abundant number of dryers on the market for drying all sorts of products. Each class of product requires its own typical dryer. The first step in selection a suitable dryer is to rank the different available dryers based on dryer feed properties, sulphur drying characteristics, and product specifications.

Dryer feed properties
A dryer is fed with sulphur pasta from the centrifuge. Typical solids concentration is 55 – 65w%. However, when viscosity is too high the solids content can be reduced. The pasta might show thixotropic flow behavior. In storage the pasta is a solid mud, but when the sludge solution is shaken it will liquefy. Otherwise the solution is a pasta that shows Newtonian flow behavior. Particle size of the sludge is less than 40 μm.

Dryer product specifications
There is in general no specific requirement towards particle size of the dried product. However, local regulation for application of the sulphur powder might require that the powder fulphill the FAO (Food and Agricultural Organization of the United Nations) particle size requirement; i.e. over 98% of the powder has to have a particle size less than 53 μm.

A small particle size is preferred when biosulphur is applied in agriculture, as a smaller particle size gives a larger total area per kg powder. Therefore, a particle size distribution of the dried equal to the feed product is preferred.

The dryness of the product should not exceed 90%. The complete dry powder is classified as a ST3 dust by TNO. This classification means that the Thiopaq™ biosulphur falls in the group of most risk full explosion materials.

Particle properties

At ambient temperature and pressure the most stable form of elemental sulphur is the crystalline orthorhombic α-S_8-state. Above temperatures of 95 °C the crystalline monoclinic form is more stable. Crystal growth is an ordered process. If the crystal growth process is hindered or the growth is not happening as an ordered process amorphous sulphur will be formed. The melting point of orthorhombic sulphur is 115 °C, and the melting point of monoclinic sulphur is 119 °C. Amorphous sulphur has a melting point of 120 °C. The density of crystalline sulphur is between 1900 – 2200 kg/m^3. Thiopaq ™ biosulphur is a mixture of amorphous and crystalline sulphur and biomass and salts. The biosulphur is hydrophilic.

Drying Characteristics

Two different drying experiments have been performed. In the first experiment sulphur was dried at 110 °C. A batch of sulphur was put in a beaker and the change of the sulphur was visually followed. Samples were taken form this beaker and they were analyzed for water content. Figure 2.1 shows the results. It follows from regression analysis that the drying rate is constant (r^2=0.94). The drying rate is 0.002 kg/kg/min.

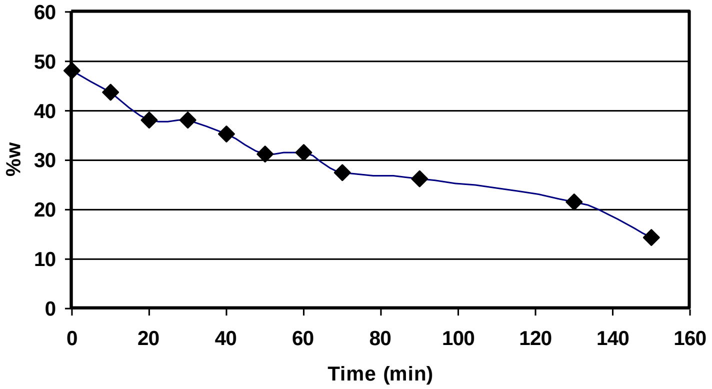

Figure 2.1. Batch drying of sulphur at 110 °C. Total weight of the sample 14.6 g. Water content as function of drying time

From visual observation the following was noticed. After 30 minutes (ca. 38w%) the product changed from a paste to lumps of sulphur hanging to the wall. Atfer 60 minutes (ca 31w%) the sulphur lumps turned into granules. These granules were free flowing. After 130 minutes (ca w21%) the product became a powder.

The results of a second dryer test are shown in figure 2.2. This figure shows the drying as a function of the water content. A wet sample of 1.7 g (60%w) was put on an infrared heated microbalance and the weight was recorded every 30s. The temperature was kept constant at 105 °C. These results were converted to a drying rate and water content of the sample. Figure 2.2 shows that up to water content of 23w% the drying rate is constant. This water content is then by definition the critical water content. Two different drying regimes can be identified: up to a water content of 23w% an equal amount of heat is required to dry the sulphur pasta. In order to reduce the water content below 23w% an increasing amount of heat per kg evaporated water is required.

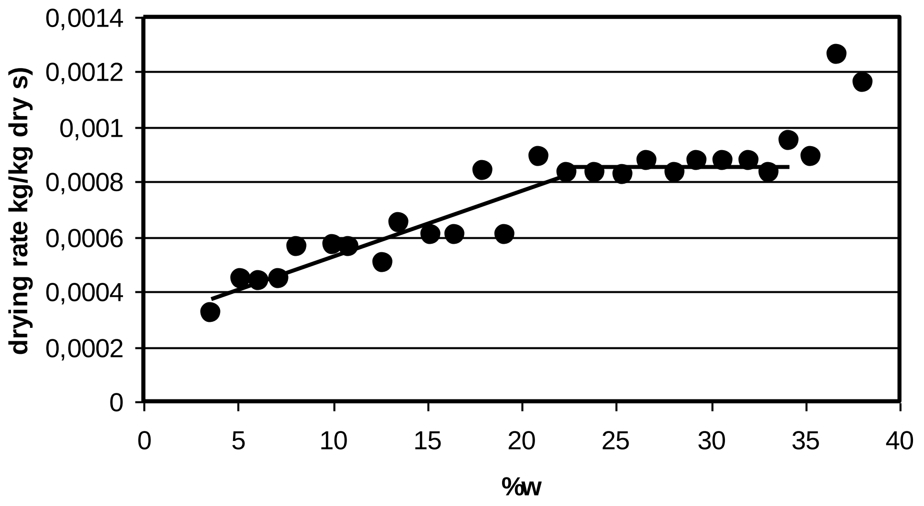

Figure 2.2. Drying rate for sulphur pasta versus water content. Drying temperature 105 °C, sample volume 1.7 g. Initial water content 60w%.

Based on the above-mentioned properties, and using an expert program the next dryers were selected:

- high speed paddle dryer
- spray dryer
- inert medium spouted bed dryer

High speed paddle dryer

A paddle dryer consist of a horizontal agitator rotating within a cylindrical housing. This housing is jacketed for indirect heating; either steam or a suitable heating liquid is used. A large number of narrow, flat adjustable radial paddles are attached to the agitator. There is a narrow adjustable gap between paddle and wall (less than 1 cm). The rotation speed of the agitator is such that the solids to be dried are kept on the cylindrical wall and they are moved forward by the action of the sweeping paddles. The residence time of the particles can be adjusted with the angle of the paddles with respect to the forward direction in the cylinder. Agglomeration of the particles is likely to occur in paddle dryers. One of the manufactures claims that with a special type of paddles the agglomerate can be pulverized. The inside surface of the solids layer on the wall assumes approximately the wet bulb temperature as long as drying is in the constant rate period. This is the case as long as the moisture content is higher than the critical moisture content. Sweep gas is needed to carry away the evaporated water from dryer. For sulphur drying this is N_2. This vent gas can be routed co- or counter currently. If routed counter-currently the solids are heated more efficiently and the temperature of the solids will be between the wall temperature and that of the inlet gas. This might be too high for sulphur drying. If the gas is routed co-currently the heating is less effectively, and the solids may leave the dryer at a temperature close to the wet bulb temperature, which is more desired for sulphur.

Paddle dryers from two different manufactures were tested. At the first manufacture a pilot plant with a capacity of 45 kg/hr was used. Two wall temperature were tested; above the melting temperature of sulphur and just below the melting temperature of sulphur. At the higher temperature molten sulphur particles were found in the product. It was concluded that sulphur drying has to be done at lower temperature.

In a new experiment the lower temperature was applied, and this test was successful. The dryer was operated for several hours without any signs of deterioration of heat transfer that would indicate jacket fouling.

The pilot plant at the second manufacture processed 110 kg/hr of sulphur. The wall temperature was well below the melting temperature of sulphur. The test run took 6 hours and it went without problems. In total 350 kg/hr of powder was produced.

Spray dryer

The basic idea of spray drying is the production of highly dispersed powders from a solution or a suspension by evaporating the solvent. In a first step the solution/suspension is sprayed into a hot air stream. The formed droplets have a large surface in comparison to their volume, and water will evaporate very effectively.

The dryer pilot plant consisted of a feeding unit, the actual dryer, a filter, a condenser, a fan and a heater. Nitrogen was used as drying gas. This gas was recycled through the drying unit. This type of dryer has a vertical cylindrical shape with a horizontal rotating dispersing wheel in the bottom. In the top is a horizontal classifying wheel. Slurry drops into the dispersing wheel and is forced out by centrifugal force into the narrow gap between the dispersing wheel and the wall of the dryer. The drying gas enters the dryer below the dispersing wheel and flows through the slurry-loaded gap between dispersing wheel and dryer wall. The slurry is broken up and atomized into droplets by the shearing of the rotating wheel and the drying gas. The small slurry droplets are dried and entrained by the drying gas towards the rotating classifying wheel in the roof of the drying chamber. This wheel contains radial vanes, and the gas together with the particles is supposed to flow from the periphery to the center of the wheel through the openings between the radial vanes. Only the smaller particles are able to overcome the centrifugal force, and they will leave the dryer with the gas. The larger, wet particles fall back through the central core and are re-entrained in the upward flowing gas stream or they fall back on the dispersing wheel. A pulse jet type of filter is used to separate the sulphur particles from the gas stream.

The pilot plant was feed with 45 kg/hr and the gas inlet temperature was well below the melting temperature of sulphur. Two different runs were performed using biosulphur from two different production plants. Both pilot plant runs were successfully operated for 6 hours, although the dryer had to be modified a lot. The filter bags were clean. The solids behavior of the biosulphur from both production plants was different. At the same solids content one plant produced a yoghurt-like liquid, while the other plant produced a dough-like paste. The test with the yoghurt-like liquid gave no problems with discharging the powder in the conical outlet of the filter, whereas the test with the dough-like paste sulphur gave stickiness problems in the conical outlet of the filter.

Inert medium spouting bed dryer

An inert medium spouted bed dryer consist of a fluid bed or spouted bed of inert glass beads of a 2-3 mm with a narrow particle size distribution. The sulphur slurry is pumped through a fluid nozzle with a diameter smaller than that of the beads. This causes the slurry to be coated onto the surface of the beads. The heat of the drying gas, N_2 , causes the coated slurry to be dried. The dried sulphur is peeled from the beads by collisions with other beads and the walls. The sulphur powder is entrained by the drying gas and separated in a filter.

A pilot plant test run was performed with this type of dryer. At an initial feed rate of 14 kg/hr and a gas inlet temperature just above the melting temperature of sulphur, the dryer performed very well without upset. The gas outlet temperature was well below the melting temperature of the sulphur. The feed rate of the dryer was increased, and the gas inlet temperature was increased. At a feed rate of 17 kg/hr the fluidisation continued to proceed smoothly, but a few beads start agglomerating. This is an indication that the maximum capacity was reached.

A second test run with the same dryer was performed over a period of 12 hours. Equal conditions were applied as in the first test run. This test run was operated at a slurry feed rate of 10 kg/hr. The test run went very smoothly.

Based on the test run performed with the different types of dryers, acceptable operating results were obtained with a paddle dryer and an inert fluid bed dryer. A spray drier did not perform satisfactorily. The texture of the powders obtained from the different dryer types, depended on the dryer: a paddle dryer produced a sand-like powder, while the inert fluid bed dryer and the flash dryer produced a flour-like powder. Thus in order to produce a powder with a small particle size less than 50 μm an inert fluid bed dryer is the preferred choice.

3. THIOPAQ™ SULPHUR: PHYSICO-CHEMICAL ASPECTS

3.1. Introduction

Sulphur is applied as a fungicide in the form of a 'dry' powder as well as a 'wettable' powder (Cooper, Resende et al. 1996; Holb and Heijne 2001). The wettable powder is dispersed in the tank of a spraying machine and sprayed on plant leaves in the form of small droplets. Wetting of the leaf surface and attachment of the sulphur particles to the leaf are important parameters in pesticidal applications and form an interesting physico-chemical problem. Characterization of the physical properties of the particles and determination of their colloidal and surface chemical properties is a prerequisite for an optimal application of biologically produced sulphur particles as e.g. a fungicide or a fertilizer.

Firstly, in this chapter an overview of the properties of biologically produced sulphur will be presented. The physical-chemical and surface properties of dried sulphur powders will be determined for different drying procedures and different Thiopaq™ installations.

Emphasis in the characterization experiments will be on the wetting behavior of the sulphur particles and on the adhesion to model surfaces and leaves, both in air as well as in aqueous environment. A novel technique has been applied for directly measuring interaction forces with model and leaf surfaces by attaching a single sulphur particle to the tip (cantilever) of an atomic force microscope (AFM). Comparisons are made with inorganic sulphur and Thiovit® Jet, a commercial formulated sulphur fungicide from Syngenta. An outlook and suggestions for further research necessary for developing a competitive fungicide based on Thiopaq™ sulphur particles will complete this chapter.

3.1.1. Colloidal stability of sulphur particles

Sulphur particles formed by sulphur compound oxidizing bacteria have particle sizes that fall in the range of the colloidal domain (approximately up to 1.0 μm) (Janssen, De Keizer et al. 1996). The internally stored sulphur particles produced by four different chemotrophic *Beggiatoas* as well as by a phototrophic *Allochromatium* bacterium have a particle diameter around 250 nm (both observed with electron microscopy) but the diameter of internally stored sulphur globules can reach up to 1 μm. Extracellularly stored sulphur globules produced by *Thiobacillus* bacteria in a sulphide-oxidizing reactor, as for Thiopaq™ sulphur, initially have approximately the same diameter (observed with electron microscopy and size distribution measured with Single Particle Optical Sizing).

The balance between attractive and repulsive forces governs the stability of colloidal sulphur particles. The most important forces are the van der Waals attraction and electrostatic repulsion, on which the DLVO-theory of colloidal stability is based. The attractive van der Waals force depends on the size of and the distance between two bodies. The repulsive electrostatic force originates from the surface charge of the colloidal particles. Apart from this, structural forces (e.g. hydrogen bonding) can stabilize or destabilize colloidal particles depending on the nature of the particles, and adsorption of polymer material at the sulphur-solvent interface can cause steric stabilization.

The origin of charge can be the presence of surface functional groups, such as carboxylic acid or amino groups. Surfaces on which proteins are adsorbed show a dependency of the surface charge on the pH value due to the presence of carboxylic acid and amino groups. At high pH a protein-covered surface will be negatively charged ($-COO^-$ and $-NH_2$ groups) and at low pH the surface will show an overall positive charge ($-COOH$ and $-NH_3^+$ groups). The pH at which the overall surface charge is zero is called the point of zero charge. In the absence of specific adsorption it coincides with the iso-electric point.

This surface charge attracts oppositely charged ions to the surface and equally charged ions are repelled from the surface. This results in an ion concentration profile as is represented in figure 3.1, which is called the diffuse electric double layer. Presence of salt causes a screening of the electric double layer.

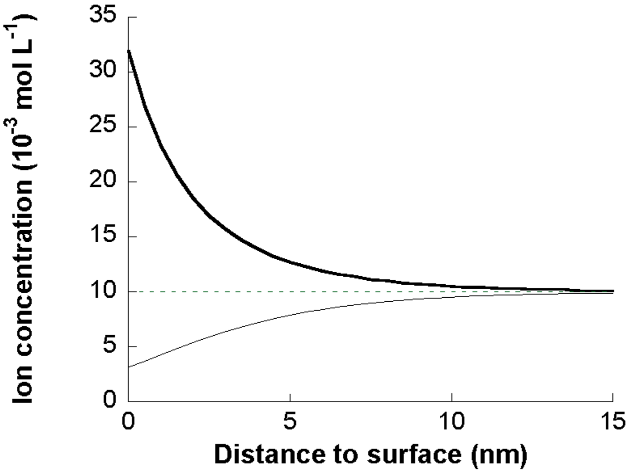

Figure 3.1. Concentration profile of positive (—) and negative (—) ions in a diffuse electrical double layer near a negatively charged surface. [NaCl] = 0.01 mol l^{-1}.

The repulsive and attractive energies of two interfaces can be calculated as a function of their distance (figure 3.2). When salt is added to a colloidal solution the electrostatic repulsion can be screened, resulting in a destabilization of the colloids.

Long chain polymers, such as proteins, can often adsorb to the surface of colloidal particles (because they gain adsorption energy), which can have a significant influence on colloidal stability. Adsorption of polymeric material at an interface takes place in such a way that the chains can extend from the surface into the solution and can rearrange their position and orientation. The effect of polymer adsorption can be both attractive and repulsive but in most cases the effect is repulsive.

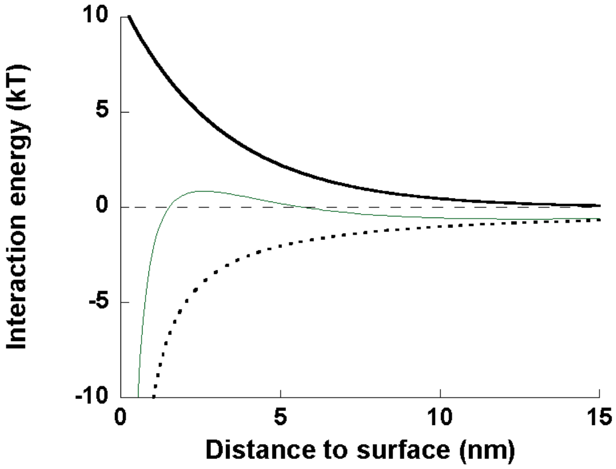

Figure 3.2. Electrostatic repulsive (—), van der Waals attractive (• • •) and total (—) interaction energies of two approaching spherical particles. Particle radius, R = 100 nm; Stern potential, Ψ_d = 10 mV; Hamaker constant, A = 0.5 10^{-20} J.

Stabilization of colloidal particles by adsorption of an uncharged polymer is called steric stabilization. The two main contributions to this influence are a volume restriction effect and an osmotic effect. The volume restriction effect is caused by the loss of configurational entropy if the adsorbed polymer chain is compressed (less possible configurations of the flexible part of the polymer). The osmotic effect is caused by the increase in osmotic pressure between two particles if the polymer layers of two particles overlap. This effect depends on the quality of the solvent. In a good solvent polymer segments favor contact with the solvent over contact with other polymer segments which causes repulsion. In a poor solvent, polymer segments favor contact with other polymer segments over contact with the solvent.

Apart from polymer adsorption of uncharged macromolecules, charged macromolecules (polyelectrolytes), such as proteins can also adsorb at surfaces. Adsorption of a charged macromolecule is different from adsorption of an uncharged polymer in that there is a high dependency on the salt concentration. At a low salt concentration, repulsive electrostatic forces between charged polymer chains will inhibit formation of loops and tails (see figure 3.3).

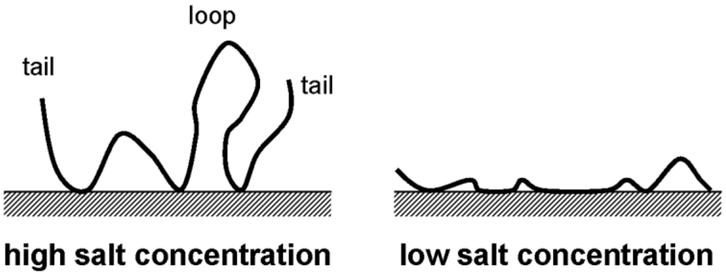

Figure 3.3. The effect of salt concentration on the adsorption of charged polymers at a surface.

3.1.2. Properties of biological produced sulphur

In the last 30 years a significant amount of research has been done on sulphur globules that are stored intracellularly (especially sulphur globules of *Al. vinosum* and *Beggiatoa alba*) and that are excreted outside the cell membrane (especially *Thiobacillus* sp.). This section is about the physico-chemical properties of those biologically produced sulphur globules stored extracellularly.

Extracellularly stored sulphur. Sulphur globules produced by bacteria excreting the globules outside the cell membrane have also been studied. Especially for biotechnological applications these sulphur compound oxidizing bacteria are interesting since they allow an easy separation of the sulphur from the bacteria. In figure 3.4 sulphur globules can be seen which have been excreted by a *Thiobacillus* bacterium.

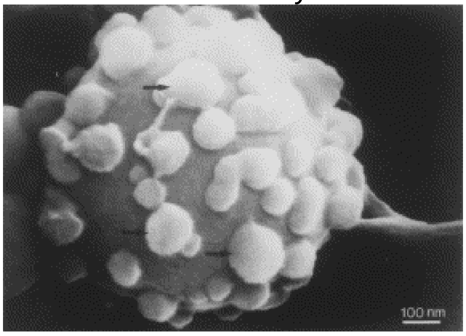

Figure 3.4. Scanning electron micrograph of sulphur excreting *Thiobacillus (Janssen, Lettinga et al. 1999)*

Steudel proposed a number of models for the composition of bacterial sulphur globules in which he did not always distinguish between intracellularly and extracellularly stored sulphur. In a model proposed for sulphur globules excreted by *Acidithiobacillus ferrooxidans* (Steudel 1996) the globules consist of a sulphur nucleus (mainly S_8 rings and small amounts of other sulphur rings) and long-chain polythionates present on the surface. Indeed HPLC and XANES analysis showed the presence of polythionates in the cultures of *Ac. ferrooxidans* excreting sulphur globules. XANES analysis did however also show that S_8 rings were not present. Another model proposed by Steudel (Steudel 1989) consists of vesicles composed of a polythionate membrane. The inside of the vesicle may contain water, and sulphur rings are only present in low concentrations in the membrane part of the vesicle. This model is in accordance with the observed experimental data obtained by XANES analysis and therefore seems suitable for globules produced by *Ac. ferrooxidans*.

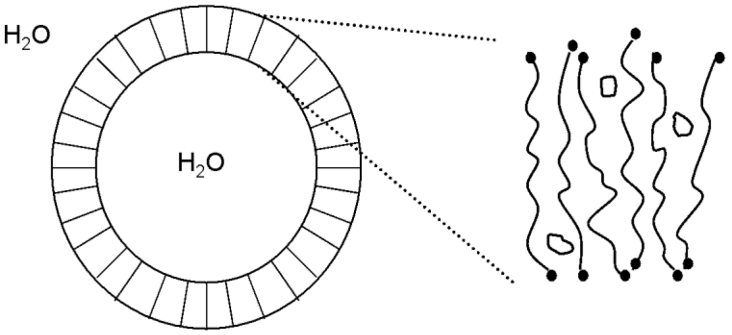

Figure 3.5. Vesicle model proposed by Steudel for sulphur globules excreted by *Ac. ferrooxidans*. Vesicles are composed of long chain polythionates ($^-O_3S\text{-}S_n\text{-}SO_3^-$) in which small amounts of sulphur rings can be present (after (Steudel 1989)).

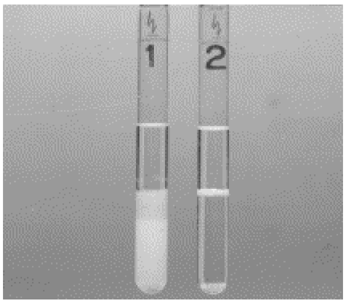

Figure 3.6. Hexadecane-water partition test. Biologically produced sulphur remains in the lower water phase (1) whereas crystalline sulphur remains in the upper hexadecane-phase (2) (Janssen, Lettinga et al. 1999).

It should be noted that polythionates are only stable at low pH (Steudel 2000) and it is therefore unlikely that the polythionate vesicle model is applicable to sulphur globules produced by bacteria growing at another pH than the acidic conditions at which *Ac. ferrooxidans* grows (see figure 3.6).

Janssen et al. have studied the properties of sulphur produced by bacteria of the genus *Thiobacillus* grown at neutral to alkaline pH (Janssen, De Keizer et al. 1994; Janssen, De Keizer et al. 1996; Janssen, Lettinga et al. 1999). The sulphur was produced in a bioreactor in which a mixed culture of *Thiobacilli* was present.

X-ray diffraction of sulphur particles excreted by *Thiobacillus* sp. showed the presence of orthorhombic sulphur crystals. The solubility of crystalline orthorhombic sulphur in water is known to be only 5 $\mu g\ L^{-1}$. In the solubility test shown in figure 3.7 it was seen that the biologically produced sulphur particles can be dispersed in water but not in hexadecane, whereas crystalline orthorhombic sulphur is soluble in hexadecane but not in water. The reason for the observed hydrophilicity of the biologically produced sulphur particles has to be attributed to the hydrophilic properties of the surface of the sulphur particles. Because of the relatively high stability of the biologically produced sulphur particles at high salt concentrations, it is concluded that the colloidal stability is not merely based on electrostatic repulsion. Hydrophobic sulphur can be wetted by *Thiobacillus thiooxidans* bacteria due to formation of organic surface-active substances.

It was concluded that long chain polymers, most probably proteins, stabilize the particles. Indeed, measurements with dynamic light scattering showed a relatively high variation of the determined hydrodynamic radius with changing salt concentration, indicating the presence of relatively thick adsorbed layers on the sulphur particles (see figure 3.3). This, as well as surface charge density measurements showing values comparable to surface charge densities of bacterial cell walls and humic acids support the suggestion of proteins adsorbed on the particles. In addition, electrophoretic mobility experiments showed an iso-electric point comparable to the pK_a-value of carboxylic acid groups in proteins (pK_a=2.3)(Kleinjan 2004).

Proteins on the surface of sulphur globules stored intracellularly act as a membrane between the cytoplasm and the intracellular sulphur particle. It is not known whether the proteins associated with the sulphur particles excreted by *Thiobacillus* bacteria are well-defined proteins synthesized by the bacterium or if they are originating from organic compounds already present in the liquid reactor system.

Formation of biologically produced sulphur. Biologically produced sulphur is often written as ‘S^0’, which only indicates that a large part of the sulphur in the sulphur globules is present in oxidation state zero. It should not be mistaken for atomic sulphur, which has a very high enthalpy of formation and therefore cannot exist at ambient temperature. In the previous sections it has been shown that the sulphur in globules can be present as sulphur rings, long-chain polythionates and long sulphur chains terminated by organic groups. The mechanisms in which these forms of sulphur are formed are not known but the formation of sulphur rings is assumed to proceed through the formation of polysulphides (Brune 1989).

Steudel (Steudel 1996) proposed a reaction mechanism for the chemical oxidation of sulphide to sulphur rings (S_8), which should take place in a similar way in sulphur compound oxidizing bacteria. In this mechanism HS^- anions are oxidized forming sulphide radicals ($HS^\bullet$ or $S^{\bullet-}$).

$$HS^- \rightarrow HS^\bullet + e^- \tag{3.1}$$

$$HS^\bullet \rightarrow S^{\bullet-} + H^+ \tag{3.2}$$

These radicals are the basis for a complex sequence of reactions involving radicalization of ions and dimerization of radical ions, resulting in polysulphide anions (S_x^{2-}), e.g.:

$$2S^{\bullet-} \rightarrow S_2^{2-} \tag{3.3}$$

$$S_2^{2-} + S^{\bullet-} \rightarrow S_3^{\bullet-} \tag{3.4}$$

$$S_3^{\bullet-} + S^{\bullet-} \rightarrow S_4^{2-} \tag{3.5}$$

$$2S_3^{\bullet-} \rightarrow S_6^{2-} \tag{3.6}$$

Etc.

Upon acidification of long chain polysulphide anions, elemental sulphur rings are formed.

$$S_9^{2-} + H^+ \rightarrow HS^- + S_8 \tag{3.7}$$

In chemical oxidation reactions, the first oxidation step (forming of sulphide radicals) is catalyzed by metal-ions like V^{5+}, Fe^{3+}, and Cu^{2+}. In most sulphur compound oxidizing bacteria, the first step in the oxidation of sulphide to sulphur is catalyzed by the enzyme flavocytochrome c (Brune 1989). In a number of bacteria with the capacity to oxidize sulphide to sulphur, flavocytochrome c has not been found and other cytochromes or quinones are believed to catalyze the oxidation of sulphide in these organisms. Brune (Brune 1989) has suggested that the location of the catalyst for the acidification of polysulphide anions to elemental sulphur determines whether sulphur is stored extracellularly or intracellularly. Van Gemerden however suggested that the location of the electron acceptor in the HS^- oxidation step determines whether sulphur is stored extracellularly or intracellularly (Van Gemerden 1984) as is explained in figure 3.7.

This is supported by research of Then and Trüper (Then and Trüper 1983) on sulphide oxidation in *Halorhodospira abdelmalekii*, excreting sulphur globules extracellularly. They showed the cytochrome c-551 to have a catalytic effect on the oxidation of sulphide and to be located on the outside of the cell membrane. In *Al. vinosum* cytochrome c-551 is located in the periplasmic space, the space between the outer cell wall and the cytoplasmic membrane, which is also the location of storage of sulphur globules.

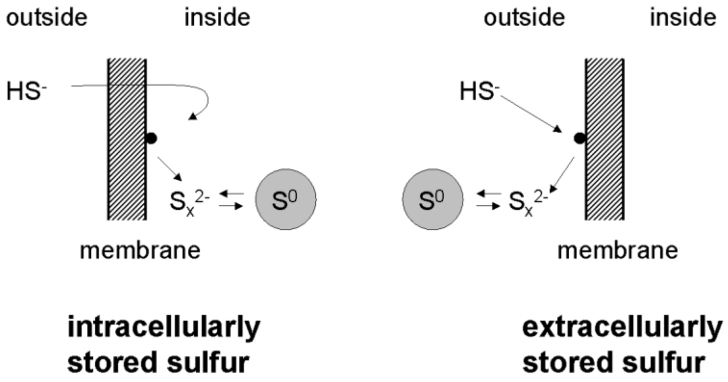

Figure 3.7. Model for the deposition of sulphur inside or outside the cells in which the position of the electron acceptor on the membrane (●) determines the location of sulphur storage (after (Van Gemerden 1984)).

3.1.3. Summary

Elemental sulphur produced by sulphur compound oxidizing bacteria ("biosulphur") has distinctly different properties than crystalline elemental sulphur. The hydrophilic properties of "biosulphur" are the most striking of these differences. As a result of this, biologically produced sulphur can be dispersed in aqueous solutions, whereas crystalline inorganic sulphur is hydrophobic and will not be wetted by an aqueous solution.

The origin of the hydrophilicity of biologically produced sulphur is however not the same for sulphur produced by different bacteria. Intracellularly stored sulphur globules produced by *Al. vinosum* consist of long sulphur chains terminated with organic groups. These organic end groups are likely to be responsible for the hydrophilic character of the sulphur globules. The hydrophilicity of extracellularly stored sulphur globules produced by *Ac. ferrooxidans* probably can be explained by the vesicle structure consisting mainly of polythionates ($^-O_3S\text{-}S_n\text{-}SO_3^-$). Adsorbed organic polymers such as proteins cause the hydrophilic properties of sulphur produced in biotechnological sulphide oxidation installations by a mixed culture of *Thiobacilli*.

Due to the small particle size and hydrophilic surface, biologically produced sulphur has advantages over other available sulphur sources in bioleaching and fertilizer applications.

3.2. Surface and colloid chemical characterization of sulphur particles

3.2.1. Introduction

In this section we will describe the material properties and the surface and colloid chemical characterization of the different sulphur samples. An overview of the Thiopaq™ sulphur samples is presented. Details of the drying procedure are presented in Chapter 2. The properties and the fabrication of the Thiovit® sample are described in some detail because this information can be important for developing a fungicide based on Thiopaq™ sulphur particles. The characterization focuses on (1) determination of particle size and particle size distribution, (2) wetting behavior of the particles, and (3) on the attachment force of the particles to different surfaces.

3.2.2. Materials

Thiopaq™ sulphur

Paques and Shell have developed technological procedures for drying biosulphur (see Chapter 2). The physical-chemical and surface properties of dried sulphur powders will be determined for different kind of drying procedures and different biosulphur sources. Four dried samples were obtained from Paques (Thiopaq1-4) and one fresh dried sample was obtained from Wilfred Kleinjan (Thiopaq5). Thus the following samples were used:

Thiopaq1.	Indirect paddle dryer (80 °C) / Eerbeek biosulphur
Thiopaq2.	Fluid bed dryer (85 °C) / Eerbeek/Europakarton biosulphur
Thiopaq3.	Indirect paddle dryer (45 °C) / Zülpich sulphur
Thiopaq4	Dispersion flash dryer (50 °C) / Zülpich sulphur
Thiopaq5.	Eerbeek Biosulphur, sample Wilfred Kleinjan, dried at the lab (42°C)

Thiovit® sulphur

Thiovit® Jet is a commercial formulated fungicide manufactured by Syngenta with sulphur as the active ingredient. It replaces the previous Thiovit® formulation of Syngenta. The following information is taken from a Power Point presentation by Syngenta.

Thiovit® Jet is formulated as a wettable powder in a granulated form with a sulphur concentration of 80 %. It is effective against mildew and mites. It is used in several crops, amongst others apple, grapes, cereals, rice etc. The formulation process of Thiovit® Jet is schematically shown in figure 3.8. In the reactor the additives and an “inorganic” sulphur liquid are mixed. In the next step formation of “microbilles” takes place by micronisation and a paste is formed, which is spray-dried forming dry granules of formulated sulphur (Thiovit® Jet Granular). The particle size distribution is very important for the efficacy of the sulphur fungicide. The best range is between 1 and 8 μm. This is illustrated in figure 3.9.

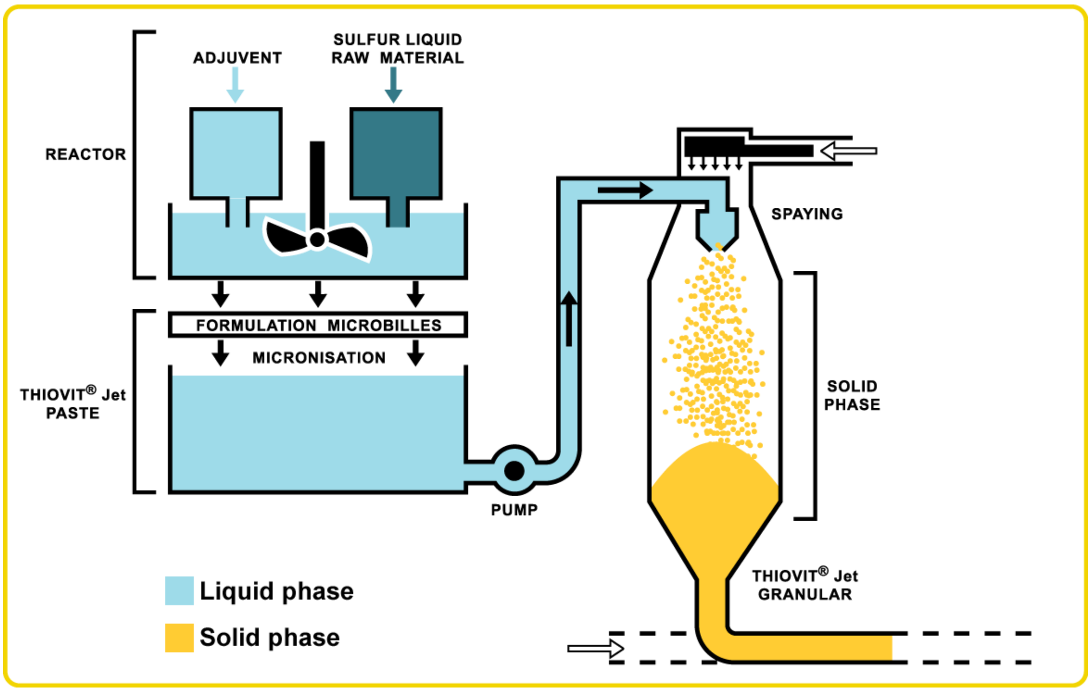

Figure 3.8 Formulation procedure of Thiovit® Jet (taken from Syngenta)

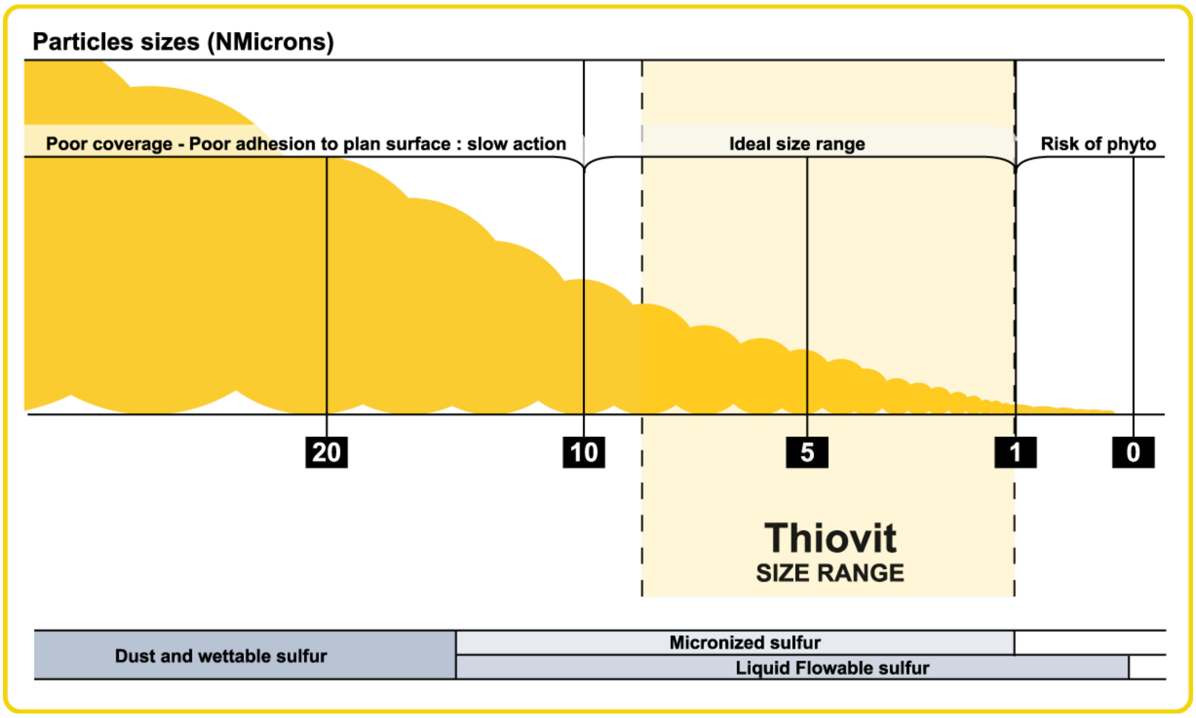

Figure 3.9 Effect of size on the efficacy of Sulphur fungicide (taken from Syngenta)

Below 1 μm the particles have a good proximity and coverage and a good contact action. Because there is a good surface of contact with air there is a good vapor action. However particles less than 1 μm can penetrate through plant stomata and 'burn' plant tissue. Moreover small particles will rapidly sublimate resulting in poor residual activity. Liquid flowable sulphur contains a small size fraction which may be phytotoxic. Therefore for particle sizes below 1μm the formulation becomes phytotoxic. Particles with sizes above 8 μm have a poor proximity and coverage and because of the small contact area with air also a poor vapor action. This is illustrated in figure 3.10a. Moreover large particles have a relatively poor adhesion leading to a low residual activity after a heavy rain or wind period. Thus dust and wettable sulphur have a relatively low efficacy. In the best size range both attachment and vapor action are good, and the sulphur particles are not phytotoxic. Thiovit Jet has an ideal particle size average of 3.28 μm and a good particle size distribution, i.e. 83% is in the 1μ - 8μ range. About 10 % of the particles is smaller than 1 μm. The fine particles of THIOVIT® Jet are coated within a larger granule (MICROSPHERE) to provide the maximum ease of handling storage and use. The THIOVIT® Jet granules contain a wetting and a dispersion agent and dissolve easily upon contact with water. In figure 3.10b the fungicidal action of an optimal formulation is schematically illustrated. The sulphur vapor is reduced to H_2S by the fungi and probably these sulphide ions kill the fungi. Thus evaporation is an important property of the sulphur fungicide. Vapor action is directly related to the specific surface area, and roughness and porosity increases the vapor action. For aggregated biosulphur particles with a diameter of about 5 μm and a relatively high specific surface area it means that phytotoxicity is absent and vapor action is optimal. Comparing the rate of evaporation for Thiovit® and Thiopaq™ would be very useful in future studies.

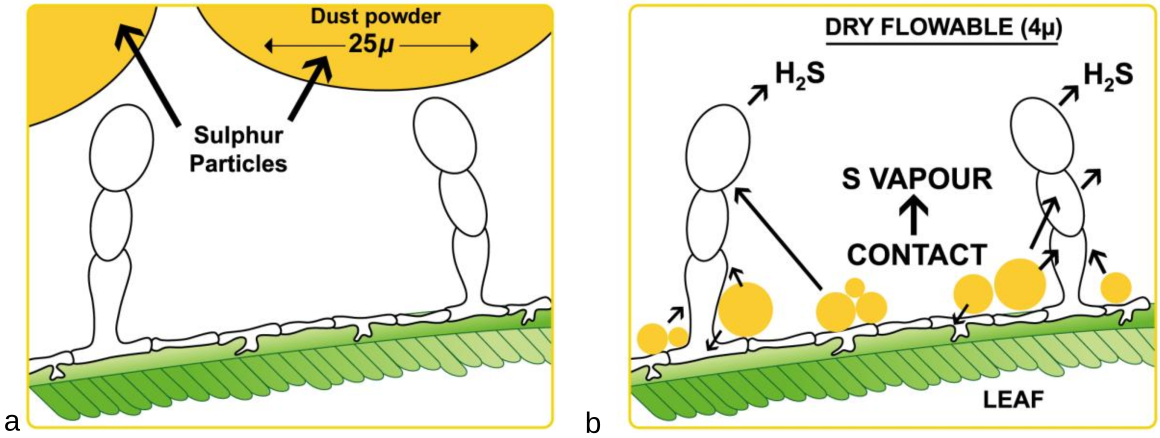

Figure 3.10 Fungicide action of sulphur particles (taken from Syngenta)

The components of Thiovit® and Thiovit® Jet are listed in Table 3.1. Compared to Thiovit® formulation the number of components in Thiovit® Jet has been drastically reduced. More dispersant (lignosulphonate) has been added. Better suspensibility and compatibility is obtained by adding $CaSO_4$.

Table 3.1. Additives of Thiovit® and Thiovit® Jet (taken from Syngenta)

Thiovit®		Thiovit® Jet	
Sulphur	80 %	Sulphur	80 %
Lignosulphonate Ca	11.6%	Lignosulphonate Ca	14.05 %
Sodium Carbonate	2.8%	$CaSO_4.2H_2O$	2.4 %
Polynaphtalene $NaSO_3$	0.3%	NaOH	1.35 %
Bentonite	2%	Water	2.2 %
Magnesium Silicate	0.6%		
Dodecylbenzene $NaSO_3$	0.1%		
Ammonium Caseinate	0.08%		
Talc	0.6%		
Water	2%		

Inorganic sulphur particles

The inorganic sulphur particles used in the wetting studies where standard yellow sulphur flower.

Model surfaces

Pellets of sulphur were formed from the dry sulphur probe particles using an IR-spectroscopy press (Anadis Press Instruments B.V.). Different pressures were applied but at higher pressures artifacts occur due to a reaction of sulphur with the steel (see section 3.2.5). Therefore in our measurements we prepared the pellets by a relatively low pressure (500 psi). Optically flat sulphur surfaces were obtained.

Flat silica surfaces were obtained by oxidizing silicon wafers to a depth of 100 μm. Before the measurements the silica wafers were cleaned in plasma cleaner (model pdc-32G, Harrick Scientific, New York, USA). This cleaning procedure results in a hydrophilic silica surface. The contact angle with water determined with KRUSS microscope was about zero degree.

Fatty alcohols (or acids) may form self-assembled highly organized monolayers on the oxide surfaces. To obtain an octadecanol-covered silica plate, the clean bare silica plate was kept in 96% alcohol during 24 hours, dried in a stream of nitrogen and deposited in an octodecanol melt at 150^{o}C for 12 hours, rinsed with alcohol and finally dried again. Under these conditions the fatty alcohol molecule is chemically attached to silica surface due to reaction of dehydration between surface hydroxyl group and the head group of fatty alcohol. The formation of such monolayer resulted in high value of contact angle (about 108 ^{o}C), confirming its high density.

An optically flat and thick model cellulose surface was made by spincoating trimethylsilyl cellulose (TMSC) on a silicon wafer. To facilitate the adhesion of TMSC to the substrate a block copolymer, polystyrene-poly(4-vinylpyridine) (PS-P4VP), was first anchored to the silicon surface. The hydrophilic PVP blocks are bound to the wafer and hydrophobic PS blocks act as anchors for the TMSC. The TMSC (20g/l chloroform solution) was then spincoated on this block copolymer layer. The TMSC layer was converted into cellulose by acid hydrolysis. The thickness of the cellulose layer measured using ellipsometry in dry air was about 100 nm (El Farissi 2004). The contact angle with water was 83 ^{o}.

Native apple leaves were chosen as an example of a plant leaf surface. Its surface is rather smooth and not very soft. An AFM image of the apple leaf is shown in figure 3.11. This enabled us to perform AFM measurements between a natural leaf and sulphur particles. Other leaf surfaces appeared too rough and too soft for making an AFM image and to perform force measurements.

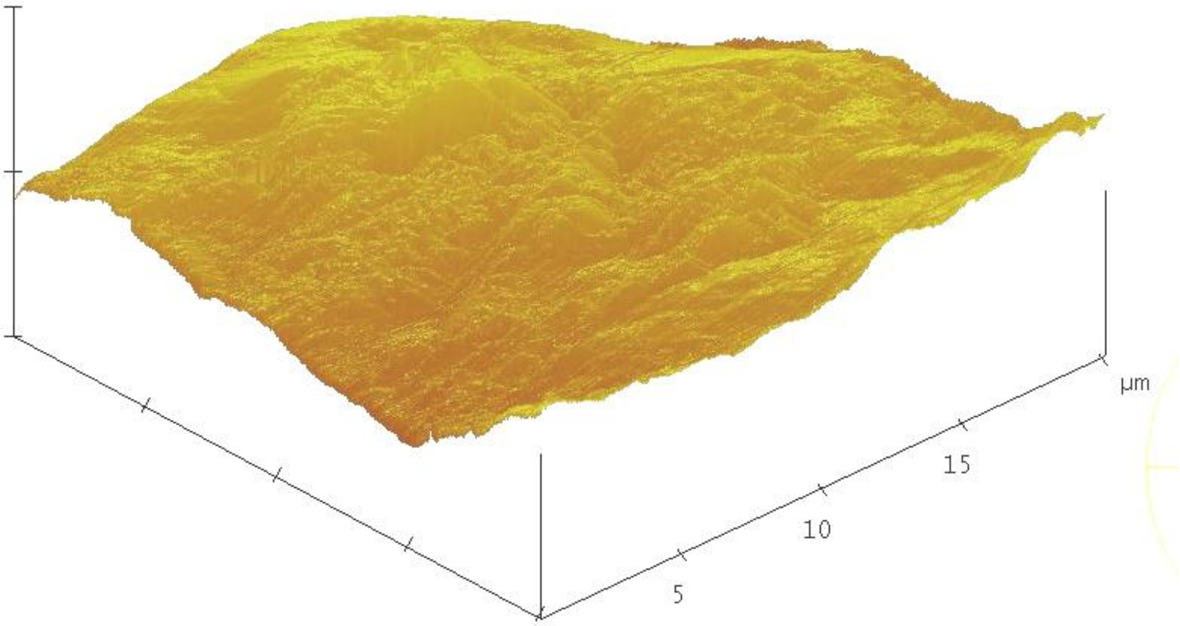

Figure 3.11 AFM image of an apple leaf surface

3.2.3. Bulk properties

Density

For several experiments (BET, contact angle) the density has to been known quite accurately. Therefore the density of the Thiopaq™ samples has been determined separately. Also the packing density of the dry powder has been measured. The pycnometrical procedure is given in detail below:
Pycnometrical procedure:

> A volumetric flask (10 ml) is weighed empty and filled with water at 20.00 ºC in order to calibrate the volumetric flask. The dry bottle is filled with dry sulphur particles up to the bottleneck. The “maximal” dry packing is obtained by ultrasonic vibration. The weight of the sulphur sample (about 10 grams) is determined with an analytical balance and the powder volume from the height of the solid particles in the bottleneck. Afterwards the volumetric flask is filled with a *surfactant* solution up to the calibration level. It is likely that wetting with water will leave air bubbles in the packed powder, whereas wetting will be strongly improved by a 0.02 M sodium dodecyl sulphate solution. The flask is filled up to volume after thermostating at 20.00 ºC and weighed afterwards. The density of the sulphur powder can then be calculated.

The density of biosulphur has been determined for Thiopaq1 and Thiopaq3 sample after drying both samples at 40ºC under vacuum. The following results were obtained

Thiopaq1	2.05 g/ml
Thiopaq3	2.01 g/ml

According to the Handbook of Chemistry and Physics the density α, β, γ - sulphur S_8 is resp. 2.07, 1.96 and 1.92 g/cm^3. Earlier, for BET-measurements we also have used 2.12 g/cm^3 (see section 3.2.4). However, for other biosulphur particles there are indications that the density of biosulphur is substantially lower (1.6 g/cm^3). However, these measurements are based on the rate of deposition of the overall particles, whereas our measurements give the density of the sulphur phase including the organic layer. Our measurements give a value between that for α- and β-sulphur.

Water content

During BET measurements it was observed that some samples were not completely dry. To that order we separately determined the water content of our samples by drying an amount of 5 grams at 40ºC under vacuum. Table 3.2 gives the results for the four Thiopaq™ samples.

The table shows a strong decrease after drying during 1 day. After 1 day it seems that per day 0.15 % is lost. We conclude that this loss is due to sublimation of sulphur. Sample 1 and 3 contain between 9 and 10 % water. Both this Eerbeek sample and Zülpich sample were dried with an indirect paddle dryer. It is surprising that the different drying temperatures have no effect on the efficiency of the drying process.

Table 3.2 Weight loss after drying biosulphur samples at 40ºC under vacuum

Thiopaq™ sample	*Weight loss after 1 day*	*After 2 days*	*After 4 days*	*water content*
1	9.3 %	0.2 %	0.3 %	9.2 %
2	0.7 %	0.2 %	0.3 %	0.6 %
3	9.5 %	0.1 %	0.3 %	9.5 %
4	1.6 %	0.2 %	0.3 %	1.5 %

Salt content of Thiopaq™ sulphur

The salt content has been determined by washing the sample and drying afterwards. For sample 3 the loss of weight was 14.7 %. The water content was 9.5 % thus the sample contains 5.2 % salt. With ICP-AES it was established that the salt is predominantly a sodium salt, most likely $NaHCO_3$.

3.2.4. Porosity, radius, and specific surface area

Specific surface area and porosity of the dry powder

Nitrogen gas adsorption at the temperature of liquid nitrogen is a standard procedure to determine the specific surface area of powders. It can be determined with the Quanta Chrome NOVA 1200 BET Surface area analyzer. The specific surface area can be obtained from the BET equation. The BET (Brunauer, Emmett and Teller) theory is based on a multilayer adsorption model and gives besides the specific surface area also a value for the affinity of the nitrogen gas for the solid (C-constant). From the BJH theory the porosity (pore volume and pore diameter) of the powder can be determined. An overview of the results is presented Table 3.3. The particle density has to be introduced separately and must be known up to 1 % accuracy. If we assume a spherical geometry of the particles the specific surface area can be easily converted to an 'equivalent' radius with equation 3.8 in which ρ [g/m^3] is the density of the particles and d [m] the diameter.

$$specific\ surface\ area = \frac{6}{\rho\, d} \qquad [m^2/g] \qquad (3.8)$$

Initially, an accurate value for the density was not available, therefore for sample 4 we have tested the dependency of the specific surface area on the density. An increase of 6 % in the density leads to a decrease of 8 % in the specific surface area. The actual density (3.2.3) is close to the assumed value of 2. Therefore, the area, C-constants and porosities at the density of 2 are most reliable. The specific surface areas vary between 0.7 and 1.5 m^2/g corresponding to an equivalent radius between 2 and 1 μm. Actually this is close to the phytotoxic limit, but one has to realise that the equivalent diameter is lower than the radius obtained by the Coulter laser (before sonification) (3.2.4). We will discuss this issue later. The lowest value for the surface area is obtained for the Eerbeek sample dried with the indirect paddle dryer at 80 ºC. Although both Eerbeek sample have a low area at is not clear why sample 1 is so low. The porosity of the Thiopaq™ samples is rather low and varies between 0.2 and 0.5 volume % and the pore diameter is around 7 nm. The C-constant indicates a moderate affinity of nitrogen gas for the sulphur surface.

Table 3.3 Determination of the specific surface area and the porosity of sulphur particles with the NOVA 1200 surface area analyzer

Thiopaq sample	*Weight Gram*	*density g/ml*	*Degas. ºC*	*S.A. m^2/g*	*Eq.diam. μm*	*C-BET -*	*Pore vol cc/g*	*Pore diam nm*
1	3.917	2.00	45	0.73	2.06	24	0.0014	7.6
2	1.816	2.00	45	1.17	1.28	52	0.0021	7.3
3	2.823	2.00	45	1.51	1.00	41	0.0025	6.6
4	2.171	2.00	45	1.33	1.13	48	0.0023	6.9

Specific surface area of the wet sulphur powder: methylene blue adsorption

Janssen et al. (Janssen, De Keizer et al. 1996) has found that the specific surface area obtained by adsorption of a dye or a polymer is substantially higher than the BET area (20-30 times higher). We anticipate that the dye binds to the organic layer and that the adsorbed amount of dye or polymer is a measure for the amount of organic material present at the surface of biosulphur particles. This information is very useful but conversion to an equivalent radius is not possible anymore. As the drying procedure can have an effect on the organic layer it seems useful to perform also adsorption measurements with a dye (methylene blue) and/or a polymer (e.g. PVA or PVP). For this purpose determination of a full adsorption isotherm is not necessary, but two values in the plateau region suffices for our purpose.

Adsorption of the dye methylene blue (MB) has been determined by depletion from solution. From the plateau values of the adsorption isotherm the specific surface area of the dispersion can be determined assuming a molecular area of MB of 1.08 nm^2 which is the value for flat adsorption of the dye molecules. For the dried powders the specific surface areas obtained were about a factor of two higher than for the values obtained with BET. If the molecules do not adsorb flat but more in a vertical position the molecular area is 0.6 nm^2. In that case the BET- and MB areas almost coincide. For native biological sulphur both Janssen (Janssen, De Keizer et al. 1996) and Kleinjan (Kleinjan 2004) found a value of around 60 m^2/g by adsorption from solution of methylene blue, paranitrofenol, and polyvinylalcohol. However, in this experiment it seems that the organic layer of the dried samples, if present, behave completely differently from the native biological sulphur samples.

Table 3.4 Comparison the specific surface area obtained with the NOVA 1200 surface area analyzer and from methylene blue adsorption

Thiopaq sample	*BET S.A. m^2/g*	*Eq.diam. μm*	*MB S.A. with 1.08 nm^2 m^2/g*	*MB S.A. with 0.6 nm^2 m^2/g*
1	0.73	2.06	1.97	1.09
2	1.17	1.28	2.30	1,28
3	1.51	1.00	1.65	0.92
4	1.25	1.15	2.95	1.64
5	2.50		59	32.8

Particle size distributions

As discussed in section 3.2.2 the particle size distribution is important parameter for the efficacy of the sulphur as a fungicide. The particle size distributions of Thiopaq™ Sulphur and formulated Thiovit® Jet Sulphur were determined with a flow ultramicroscope Coulter Laser LS 230. The particle size distribution was obtained from the light scattering of the particles according to the Fraunhofer model. For the refractive index the value of $\alpha-S_8$ sulphur (1.998) was used. Large particles were removed by passing a suspension over a Millipore filter. In order to obtain the required scattering intensity a 50 % sulphur suspension was diluted. The results for the Thiopaq™ Sulphur samples are presented in figure 3.12. The samples are rather polydispersed consisting of 3 or 4 fractions. The size of the different fractions (in

μm) is given in these figures. Only sample 4 has a big single peak with a very small minor fraction. The major sulphur fractions have diameters around 15 μm for the Eerbeek samples and 12 μm for the Zülpich samples. A minor fraction with around 200 μm is present except for sample 4. These values are much higher than obtained by gasadsorption measurements. It seems that small and large aggregates are present in suspension.

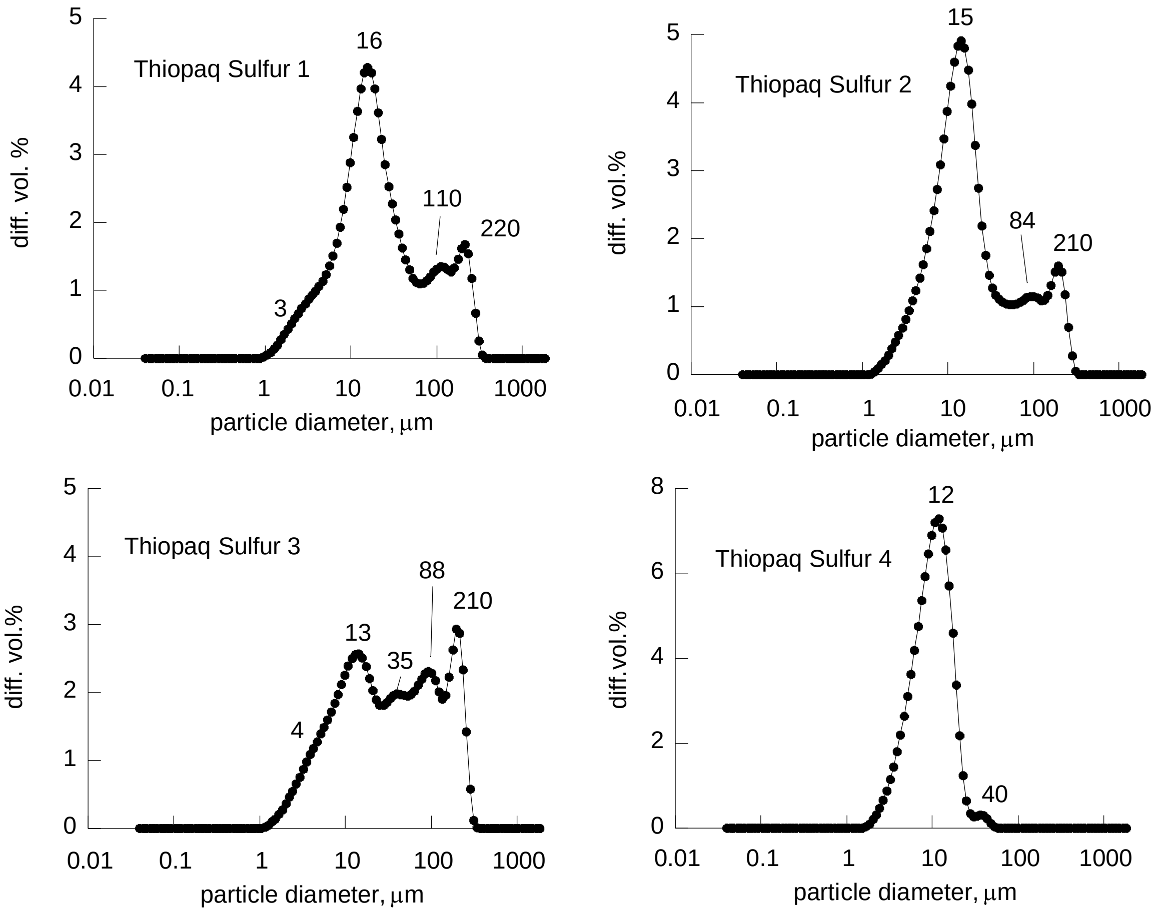

Figure 3.12 Particle size distribution of Thiopaq™ Sulphur samples. Peak positions of the different fractions are indicated in µm.

Particles or aggregates smaller than 1 µm are absent. In order to check if particles are aggregated or not, we have compared the size distribution for Thiopaq™ Sulphur 4 before and after ultrasonic treatment during 15 minutes (Figure 3.13). The size of these particles is reduced from about 10 µm to about 1-2 µm. It can be concluded that initially aggregates are present in solution. The forces between the primary particles are sufficiently low to be broken by ultrasonic vibration. The size of the particles after ultrasonic treatment corresponds with the size obtained by nitrogen adsorption. Thus the surface areas measured with BET correspond to the areas of the primary particles in the aggregates.

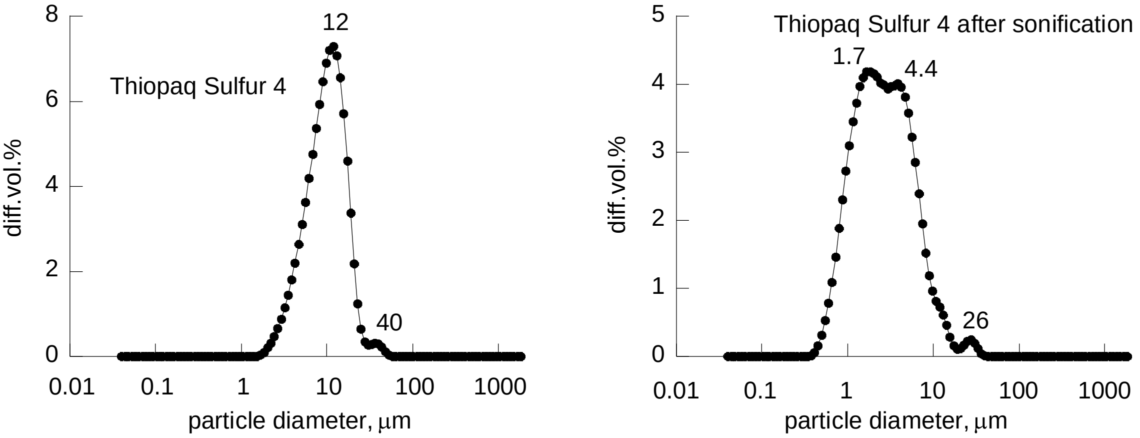

Figure 3.13 Particle size distribution of Thiopaq™ Sulphur 4 before and after ultrasonic treatment. Peak positions of the different fraction are indicated in μm.

Particle size distributions have also been determined for Thiovit® particles. Results before and after sonification are presented in figure 3.14. Before sonification the distribution is bimodal, the major fraction having a particle diameter of about 5 μm and the minor fraction about 50 μm. After sonification the minor fraction of big particles almost disappeared. Size reduction of the major fraction is almost absent for the Thiovit® sample which means that these particles are better dispersed than the biological sulphur samples. However, the primary particles are much smaller for biological sulphur than for Thiovit potentially leading to a better vapor action.

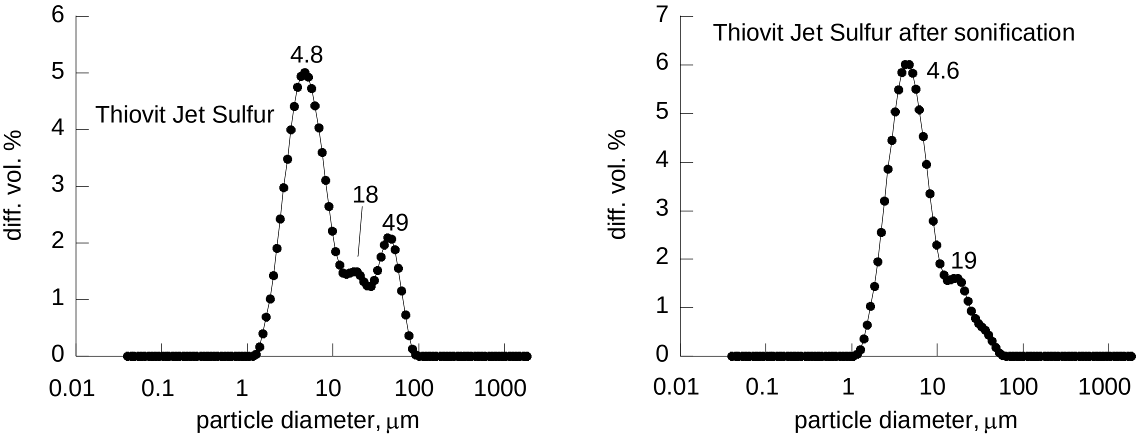

Figure 3.14 Particle size distribution of Thiovit® Sulphur before and after ultrasonic treatment. Peak positions of the different fractions are indicated in μm.

3.2.5. Surface characterization

AFM images

Images of the sulphur surface of a pellet obtained with an IR-press (see section 3.2.2) were obtained with an atomic force microscope (AFM). In the following paragraph we present a short description of the AFM microscope (Giesbers 2001).

The atomic force microscope consists of a piezoelectric scanner, a tip, a laser, a detection system and a computer (see figure 3.15). The scanner moves the sample in three directions at sub-nanometer resolution. The resolution of the images is determined by the size and geometry of the tip. The standard AFM tips are made from silicon nitride, has a pyramidal shape, about 5 μm at the basis and with a tip apex radius of curvature about 10 nm. The tip is mounted at the end of a cantilever, which is coated on the back with a reflective (gold) layer. The most commonly used cantilevers have a V-shape, making them less sensitive to lateral twisting when the tip scans the surface. The basic mode of operation of the AFM is called contact mode: the cantilever is deflected upwards and it exerts a spring force to the sample surface. The force can be as low as 10^{-9} Newtons. During imaging the deflection, and thus the force on the cantilever, can be kept at a constant value by adjusting the z-position of the piezo element. The z-movement of the piezo reflects the topography of the sample. This is the standard way of imagin in th AFM. Lowering the applied force leads to a better image resolution and reduces the chace of damaging the sample. In the tapping mode the tip is not constantly in contact with the surface but touches the surface with a certain frequency during movement of the piezo.

AFM measurements in the contact mode were performed for Thiopaq™ dried sulphur samples, a native biological sulphur (Eerbeek, obtained from Kleinjan) and crystalline sulphur (Figs. 3.16-3.19). The surfaces were obtained with an IR-press at 500 psi (35 atm). Although the surfaces are optically very flat the AFM picture clearly shows separate particles and a characteristic topographical picture of the surface. The scales for the horizontal and vertical axes are only slightly different (often scales are substantially different). In the z-direction the scale is 2 μm per division, for the x,y-axes the scale is 5 μm per division. The order of magnitude of the particle size from the AFM-image corresponds satisfactorily with that of the Coulter laser method. The biosulphur samples are rather similar with a moderate crystallinity, however the crystallinity of the crystalline inorganic sulphur is much higher.

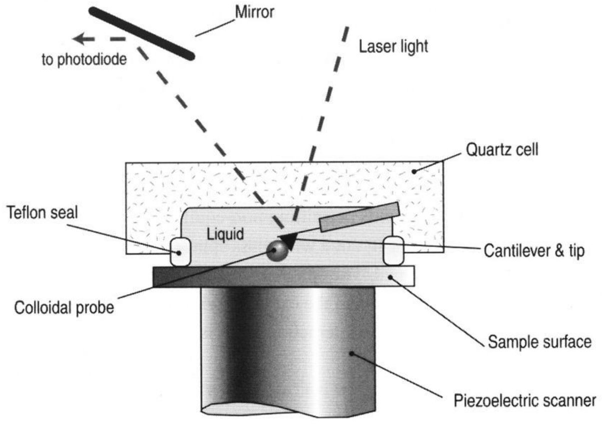

Figure 3.15 Schematic picture of an AFM (including colloidal probe)

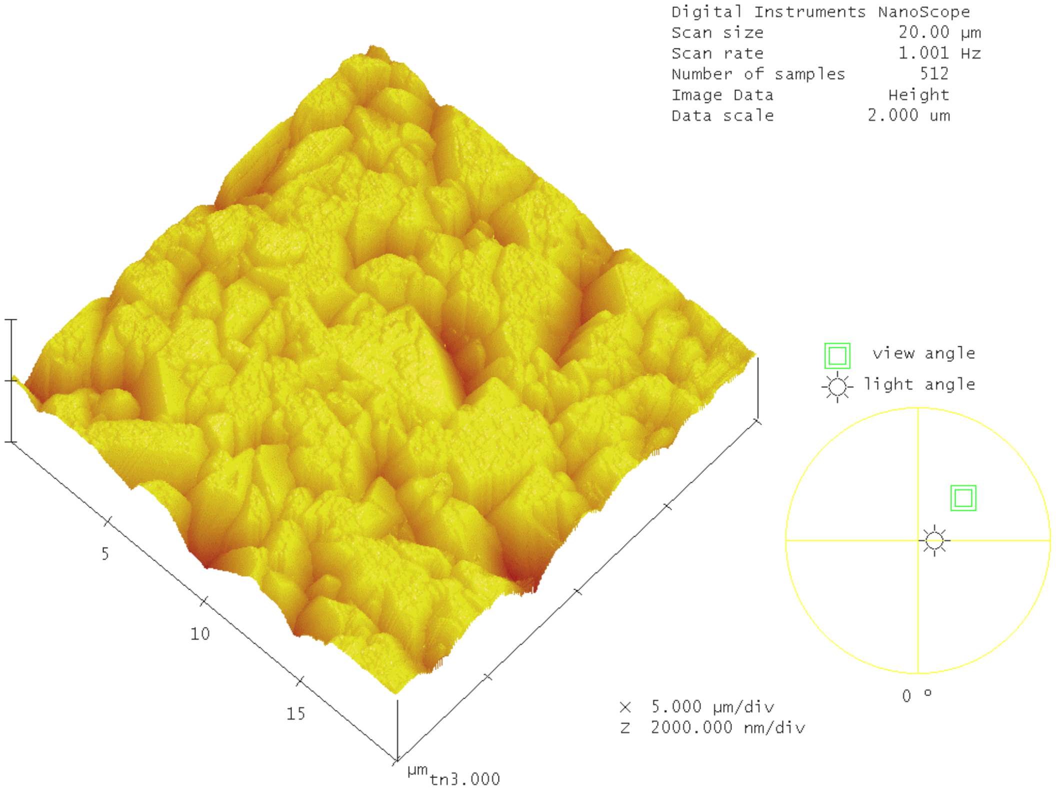

Figure 3.16 AFM image of the surface of a IR-press pellet of Thiopaq™ sulphur 3

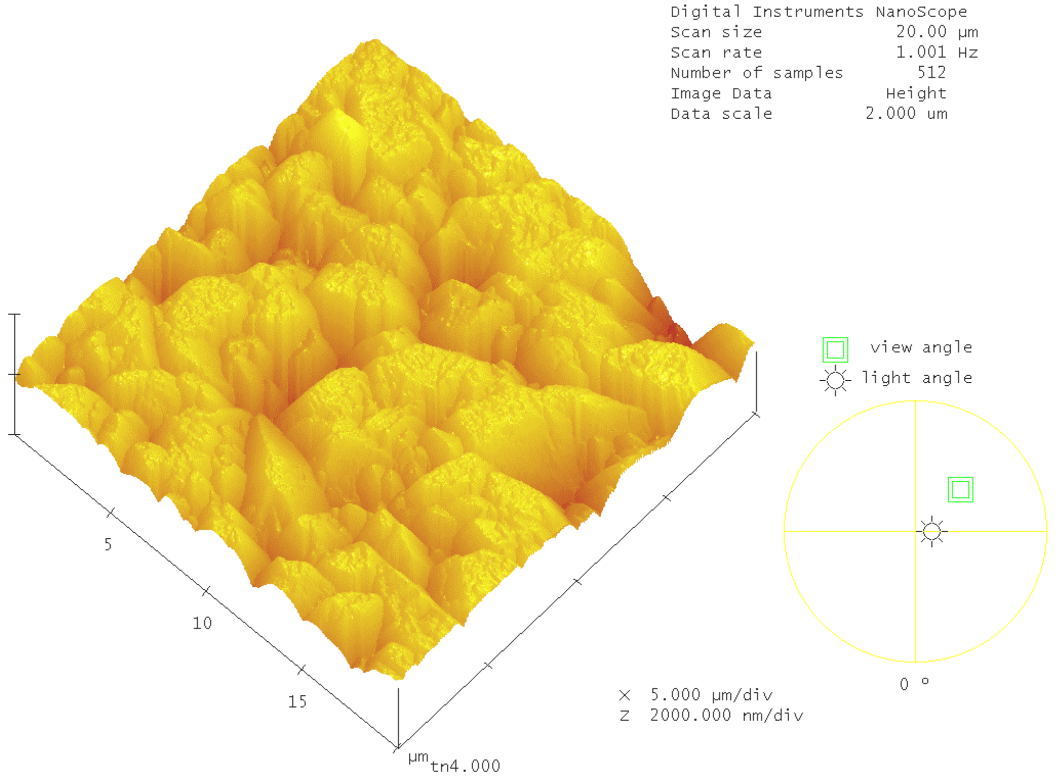

Figure 3.17 AFM image of the surface of a IR-press pellet of Thiopaq™ sulphur 4

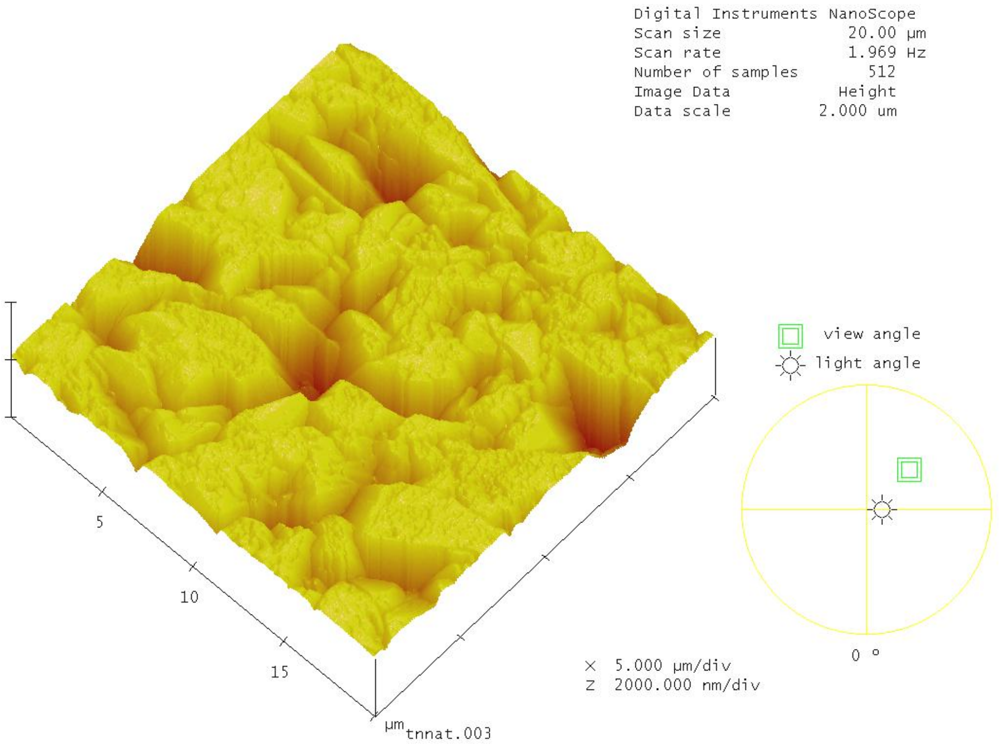

Figure 3.18 AFM image of the surface of a IR-press pellet of native biosulphur

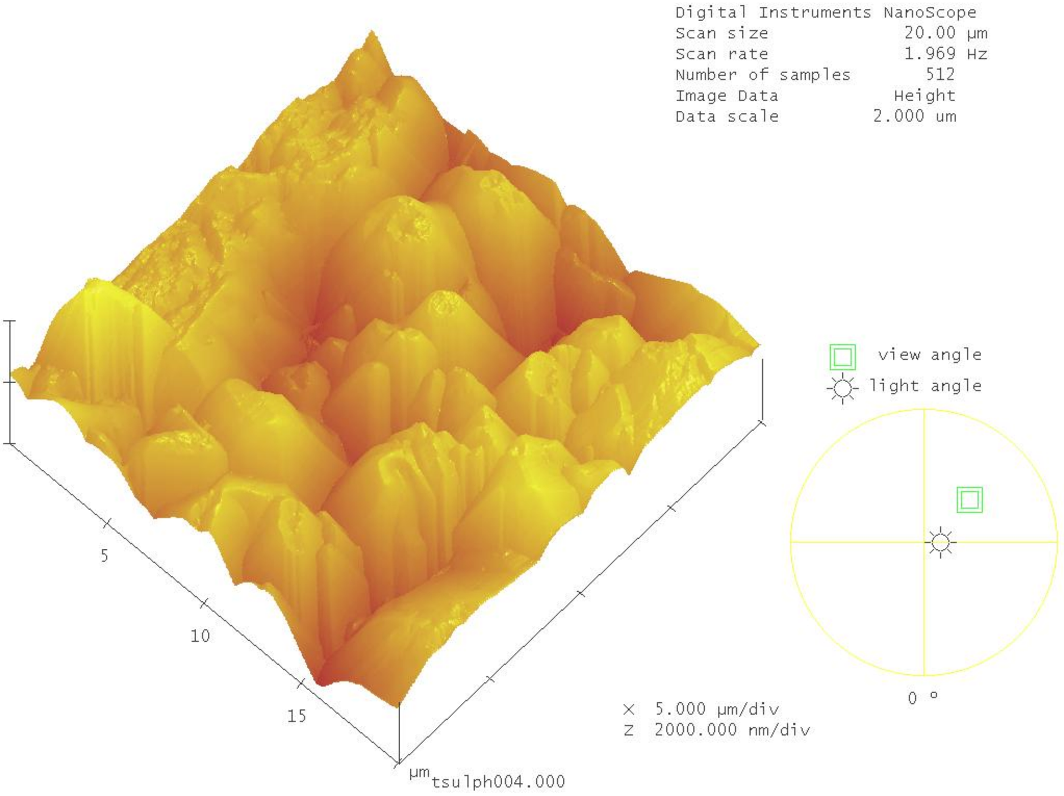

Figure 3.19 AFM image of the surface of a IR-press pellet of crystalline inorganic sulphur

Surface charge of the suspended particles

The ζ-potential of Biosulphur particles was measured with the Malvern Zetasizer 2000. In principle also the surface charge can be calculated from the ζ-potential. Measurements were performed at pH values 3 and 7. The ζ-potential at pH≅7 is almost –50 mV which corresponds to a surface charge of 0.8 $\mu C/cm^2$. Thus both the native surface as well as the dried sample carry a substantial negative charge. The charge is strongly pH dependent; at pH=3 the ζ-potential is small and for the native sample it even becomes positive. The charge of the particles must be attributed to the organic layer surrounding the particles. From the pH dependence it follows that the charges are mainly carboxylic (COOH-) groups, but also amine groups could be present, giving rise to charge reversal at pH=3. It is surprising that the drying process has a strong effect on the wetting behavior of the particles, whereas the effect on the surface charge is almost absent. The Thiopaq™ samples 1 and 3 are rather hydrophobic on one hand but carry a substantial negative charge. Probably this can be explained by the strong heterogeneity of the particles.

Table 3.5 . Electrophoretic mobilities and ζ-potentials of biosulphur particles measured in 0.005 M NaCl solution.

Biosulphur sample	pH	Mobility ($10^{-8}\,m^2V^{-1}s^{-1}$)	ζ-potential (mV)
Thiopaq™ 1	6.96	-3.90	-49.0
Thiopaq™ 1	3.12	-0.17	-3.2
Thiopaq™ 3	6.9	-3.85	-48.7
Native	6.9	-3.56	-45.3
Thiopaq™ 1	3.12	-0.17	-3.2
native	3.12	0.50	6.4

Contact angle measurements

The colloidal properties of the biologically produced sulphur particles (surface charge, electrophoretic mobility, etc.) have been discussed in section 3.1.1. It was assumed that the biosulphur particles are covered with negatively charged biopolymers. The biopolymer film is the cause of the higher hydrofilicity compared to crystalline sulphur. Janssen et al. (Janssen, De Keizer et al. 1994) has shown that the partition between a water and a hexadecane phase was remarkably different between these kinds of sulphur. In order to check if the drying process has affected the wetting behavior of the sulphur particles both microscopic contact angle and capillary rise measurements were carried out.

Contact angle measurements on a flat substrate were performed with a Krüss contact angle microscope G1 (Kruss GMBH, Germany). Measurements were performed with surfaces of sulphur pellets prepared with an IR press (section 3.2.2). Often equilibrium of the contact angle is not obtained but the measured values are different when the drop volume is increased (advancing contact angle) or decreased (receding contact angle). This difference can be attributed to heterogeneity or roughness of the surface. From the first results it became clear that the receding contact angle was always much smaller than the advancing contact angle and that the reproducibility of the receding angle was low. Therefore in our study we only report advancing contact angles. One has to be aware that these values are always higher than the equilibrium contact angles. Nevertheless interesting conclusions can be drawn from the comparison between different sulphur systems.

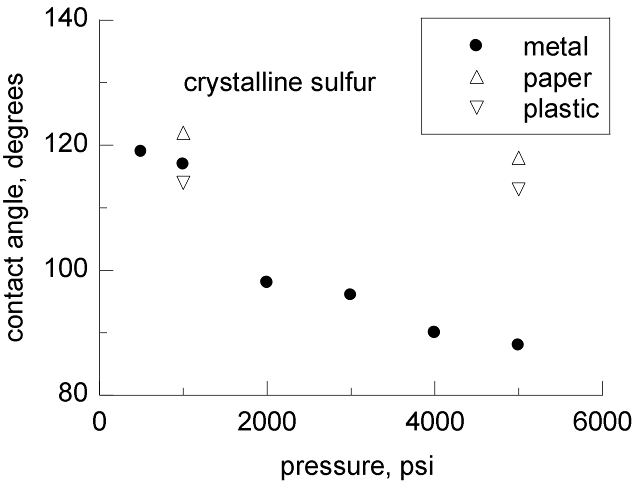

Figure 3.20 Microscopic measurement of the contact angle between water and a surface of crystalline sulphur prepared at different pressures, and with and without contact with the steel surface of the press

After some initial experiments it appeared that the resulting advancing contact angle was a function of the pressure applied during preparation of the pellet in the IR-press. As the origin of this effect could have a relation to the biofilm at the surface, we first investigated the pressure effect for crystalline sulphur. In order to distinguish between a pressure effect and an effect of the contact of steel with the sulphur, pellets were prepared by separating the steel and the sulphur with a plastic (polyethylene) film or with a paper sheet. The results are shown in figure 3.20.

In the pressure range between 500 and 1000 psi (35-70 atm) the contact angle keeps nearly constant but with increasing the pressure further the contact angle decreases. By visual observation it was detected that upon increase of the applied pressure the surface of the sulphur pellet becomes darker. It is well known that sulphur shows rather high binding energy with metals. The chemical reaction between the sulphur and the metal surface might cause the contact angle to decrease by formation of metal sulphides. To prevent this chemical reaction the paper or the plastic film was put in between sulphur and the metal surface before the pressure was applied. The results show that the pellets formed at 1000 and 5000 psi in the presence of these films give nearly the same contact angle as the sulphur pellet formed in contact with the steel surface at 1000 psi. Some differences in the values obtained at 1000 psi might be attributed to differences in surface roughness.

The same set of contact angle measurements was performed for dried microbiologically produced sulphur particles Thiopaq 2. Before the pellet was formed the sample was dried at 40^{o}C in vacuum and then the pellet was formed in contact with metal surface. The results are shown in figure 3.21. The contact angles versus pressure show the same trend as for inorganic sulphur but the absolute values of contact angle are lower. On the basis of these results and to minimize the risk of chemical reaction between sulphur and the metal surface the contact angle of biosulphur samples was measured on pellets formed at 500 psi.

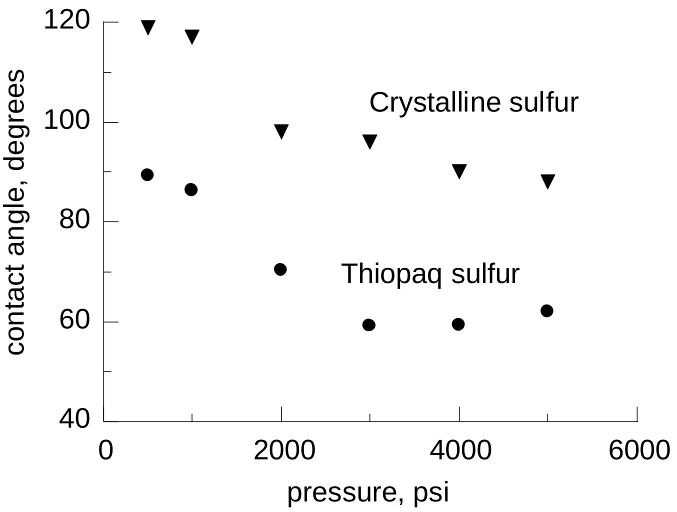

Figure 3.21 Microscopic measurement of the contact angle between water and a surface of crystalline sulphur and Thiopaq sulphur 2 prepared at different pressures

The results for all samples studied are summarized in Table 3.6. Thiopaq samples 2 and 4, were dried before the pellet formation because of the high water content. To check the effect of salts ($NaHCO_3$) the Thiopaq samples 2 and 3, and the native Eerbeek sample 5 were washed several times with deionized water until the conductivity was about that of water and then dried at 40°C in vacuum. As it follows from the results, preliminary drying or washing procedures did not substantially affect the wetting of the pellets. Thiopaq 3 and native sample 5 show considerable water penetration into pellet. A reliable contact angle could not be obtained for these samples and we must conclude that the contact angle is much lower than 90° and the samples have a hydrophilic character. As expected crystalline sulphur is very hydrophobic and a contact angle of 119° was obtained. The Eerbeek Thiopaq samples 1 and 2 are moderately hydrophobic with a contact angle of about 90°. The Zülpich sample Thiopaq 4 is slightly more hydrophilic than the Eerbeek samples. Although the contact angle of samples 1, 2 and 4 are much lower than that of the crystalline sulphur it still is much higher than expected from the partition experiment performed with the native Eerbeek sulphur. It seems that the drying procedure has affected the wetting behavior of the sulphur particles. We will return to this issue in the next section

Table 3.6. Contact angles measured between of water at a sulphur surface. The sulphur pellets were formed at 500 psi.

Sample	Thiopaq 1		Thiopaq 2			Thiopaq 4		Cryst. S
	original	dried	original	dried	washed	original	dried	original
Contact angle	91.0	90.3	89.5	89.3	90.0	86.2	84.6	119

Capillary rise measurements

In the microscopic determination of the contact angle the roughness of the pellet surface and the deformation of the adsorbed biopolymer layer under the applied pressure might affect the wetting behavior of sulphur. To eliminate these effects we used the effect of capillary penetration of water into a porous plug of sulphur particles.

The degree of wetting of a surface, or the contact angle, also follows from the rate of penetration of water into a cylindrical capillary. In the capillary the curved meniscus results in a pressure difference over the meniscus according to the Laplace equation:

$$\Delta P = \frac{2\gamma \cos\alpha}{R} \qquad (3.9)$$

Here γ is the surface tension between water (or the solution) and air (in N/m), α the contact angle and R the radius of the capillary. The Laplace pressure is counteracted by an increasing hydrostatic pressure, $\Delta\rho gh$, in which $\Delta\rho$ is the difference in density between water and air, g is 9.81 m^2/s and h is the capillary rise at a time t. Washburn derived an equation for the rate of penetration in a vertical cylindrical capillary (Eq. 3.10):

$$v = \frac{dh}{dt} = \frac{R^2}{8\eta h}\left(\frac{2\gamma \cos\alpha}{R} - \Delta\rho g h\right) \qquad (3.10)$$

Important is that the rate of penetration as a function of time depends on the contact angle α and thus the contact angle can be derived if all other parameters are known. If an open cylindrical vessel is filled with a fine powder the pores between the fine particles also results in capillary penetration. Strictly speaking the radius of the pores is not well-defined, but an effective radius can be obtained by calibration with an aqueous solution with ideal wetting i.e. a contact angle zero, e.g. a surfactant solution.

From Eq. 3.10 it follows that the rate of penetration becomes zero if the terms between brackets cancel. In that case the capillary rise is maximum and follows from Eq. 3.11.

$$h = \frac{2\gamma \cos\alpha}{\Delta\rho g R} \qquad (3.11)$$

Thus, he contact angle can also be derived from the maximum capillary rise. In order to determine the degree of wetting of a sulphur powder, a cylindrical tube with a diameter of 1 cm was filled with sulphur particles. The bottom of the tube was close with a plug of paper filter with a height of 1 cm. The bottom was placed in water and the capillary rise after 1 hour was determined. To achieve the maximum packing density the sulphur dispersion, after being deposited into the tube, was gently shacked by hand during 15 min. As we are mainly interested in the qualitative effect, we have not carried out separate calibrations with a surfactant solution. In order to remove loosely attached polymers or salts, measurements were also performed after washing the samples. The results are given in Table 3.7.

Table 3.7 Determination of the capillary rise of different sulphur powder samples after 1 hour.

sample	capillary rise, mm	
Thiopaq 1 orig	0,5	1
Thiopaq 2 orig	2	3,5
Thiopaq 2 washed	2	5
Thiopaq 3 orig	7	8
Thiopaq 3 washed	8	9
Thiopaq 4 orig	5	7
Native biosulphur 5		27
Crystalline sulphur		0

No capillary rise was detected for crystalline sulphur, which corresponds with the contact angle larger than 90° found with the microscopic measurements. The highest rise was found for the native sample (27 mm) which indicates that native biosulphur is rather hydrofilic. A relatively small capillary rise (less 5 mm) was found for the Eerbeek Thiopaq samples 1 and 2. The effect of washing was negligible. It seems that the Zülpich samples (Thiopaq 3 and 4) are more hydrofilic than the Eerbeek samples. This was also found by the microscopic contact angle determinations.

The most important finding from our capillary rise measurements is that there exists a substantial difference between native and dried biological sulphur. Thus we must conclude that the drying procedure makes the biosulphur particles much more hydrofobic. However, it is still not completely clear what are the reasons for this change in wetting behavior and is a subject of future investigation.

3.3. AFM force measurements of sulphur particles with model surfaces and plant leaves

3.3.1. Introduction

The standard AFM device (section 3.2.5) can also be used for measuring the force between the tip and a flat surface as a function of their separation. Recently, the force between a surface and a spherical particle (a colloidal probe) attached to the tip of the AFM has been measured (Giesbers 2001). In figure 3.15 a sketch of a colloidal probe attached to the tip was presented. The forces between such a colloidal probe (diameter in the order of a few μm) and a flat surface are much larger than between the tip and a surface, and therefore can be measured quite accurately. Moreover, the use of a colloidal sphere, of which the surface chemistry and geometry are known, enables comparison of experiment and theory.

The raw interaction data obtained by the AFM consist of the measured deflection of the cantilever as a function of the z-position of the piezo. The deflection of the cantilever and thus the force between the particle and surface is monitored by recording the position of the laser spot, which reflects from the back of the cantilever, on the photodiode. The photodiode output (in Volts) is converted into the deflection of the cantilever in z-direction. To achieve this, in the raw data the region is found where the photodiode signal is a linear function of the piezo position. In this region the particle on the cantilever is in contact with the sample surface on the piezo. Changes in the displacement of the signal are then equal to the changes in the deflection of the cantilever. The slope of the photodiode output *vs* piezo position in this so-called *constant compliance* region gives the conversion factor to obtain the deflection in nanometers. Provided that the cantilever spring constant is known, for each point of the raw data curve, the interaction force can be calculated using Hook's law. Zero deflection is taken from the region where the particle and surface are far apart and the photodiode output has a constant value. The distance between the particle surface and a flat surface is not measured directly but can be obtained by establishing a point of zero separation. The starting point of the constant compliance region when the surfaces are approaching each other is taken as a point of zero separation.

The force measurements described here were performed using DI (Digital Instruments Inc., Santa Barbara CA, USA) NanoScope III atomic force microscope equipped with a standard fluid cell and a piezo scanner 3549jv (x, y range 125 μm x 125 μm).

3.3.2. Attaching particles to the AFM cantilever

A crucial step in the procedure is to attach a sulphur particle to the cantilever. To that order a sharp tungsten wire is used to put glue on a cantilever tip. The colloidal particles were glued to the cantilever using an epoxy resin, Epikote 1004 (Shell, Amsterdam, The

Netherlands). This epoxy resin melts at about 100^{o}C and is highly insoluble in water. All measurements were done with “wide-legged” cantilevers 100 μm long, which have the highest spring constants (*k*=0.58 mN/m). The attachment of a colloidal particle to the cantilever tip is performed under an optical microscope and using a micromanipulator as follows. The tungsten wire (0.25 mm diameter) is etched by immersing one end in 1 M KOH solution and applying a voltage of about 40 volts between the wire and the platinum electrode placed in solution. The tungsten wire is etched until the end is about 1 μm thick. After etching the wire is rinsed with ethanol, dried in a stream of nitrogen and placed in the micromanipulator.

Some epoxy resin dust is sprinkled onto a glass slide, which is placed on the heating stage under the microscope. The heating stage is kept at a temperature of about 100^{o}C, sufficient to melt the glue particles. The sharp wire is dipped into one of the small glue droplets on the glass slide and some glue will attach to it. To reduce the amount of glue a clean part of the glass slide is touched several times with the wire until only a tiny amount of glue is left. Now, the cantilever is placed on the heating stage and touched with a glue-loaded wire on the spot where the particle will be placed. In this way a bit of the glue is transferred from the wire to the cantilever.

The next step is to bring a small number of colloidal sulphur particles onto a clean glass slide under the microscope. The average diameter of particles used was between 5 and 8 μm. The chosen sulphur particle is picked up with a sharpened and cleaned tungsten wire. This is possible because of the adhesion between the sulphur particle and the metal wire. Next, the particle is carefully manoeuvred onto the glue on the cantilever where it sticks. Even though the particles were not spherical and their surface was not smooth we managed to glue them to the cantilever tip using epoxy resin. Finally, the cantilever is removed from the heating stage, and the glue is hardened.

3.3.3. Cantilever spring constant

To calculate the forces between the colloidal probe particle and the flat surface in AFM force measurements Hook’s law is used:

$$F = - kz \qquad (3.12)$$

where F is the force, *k* is the cantilever spring constant, and *z* is the deflection. The cantilevers used in this work were provided from Digital instruments (Santa Barbara, USA). The cantilevers are mounted on a chip. Four types of cantilevers are provided on one chip: 100 μm long *wide-legged*, 100 μm *narrow-legged*, 200 μm long *wide-legged*, and 200 μm long *narrow-legged*. Digital Instruments specifies the spring constant of the various cantilever types, but the values provided indicate only the order of magnitude. For accurate force measurements and comparison with theory the spring constant of each individual cantilever has to be determined precisely.

Several methods to obtain the cantilever spring constant were proposed. They are based on application of a known force to the end of cantilever, or on the determination of the resonance frequency of both an unloaded cantilever and that loaded with a particle of known mass (Cleveland method). There are also several attempts to calculate the spring constant of the cantilever from its shape, dimensions, and material properties. The interaction force between the sulphur probe and the different surfaces was always big so that in all experiments the 100 micron wide legs triangle cantilevers with the highest spring constant of 0.58 N/m were used.

During force measurements with AFM, the tip with colloidal probe and a surface are continuously brought into contact and separated again. The piezo element moves only in the z-direction, whereas *x* and *y* positions are fixed. A scan rate of 0.6 Hz was used, for a *z* range of about 1 μm. Forces between the surfaces cause the cantilever to deflect. The monitored deflection of the cantilever is plotted versus the piezo position. Figure 3.22

shows the interaction upon approach and retraction respectively. At large distance (I) there is no force acting between the surfaces. Upon approaching the surface the particle experiences either attraction or repulsion, which causes the cantilever to bend towards or away from the surface (II). When the particle and flat surface comes into contact, the particle movement complies with the movement of the piezo (III). In this region the measured deflection is a linear function of the piezo movement. This part of the curve is *called constant compliance region*. At the end of this region the piezo movement is reversed. On retraction the surfaces are in contact beyond the point where the initial contact was made if there is an adhesion force between the surfaces (IV). A force is needed to separate them (deep minimum). Upon further separation the force between the surface and the probe becomes zero again. We already mentioned before that the deflection of the cantilever is detected by a split photodiode. The output of this photodiode is a difference between the voltage output of its top and bottom segments. At large separations the voltage output of the photodiode it constant and this value is taken as zero level of deflection and force. This zero level voltage output is subtracted from the output voltage for each data point. To convert the resulting voltage in deflection in nm, the constant compliance region is used, where the piezo and the probe movement are the same. Therefore, the slope (V/nm) of the constant compliance line provides the relation between the photodiode output in Volts and the cantilever deflection in nanometers. By multiplying the deflection with the spring constant the interaction force (in Newtons) between the particle and the surface can be obtained.

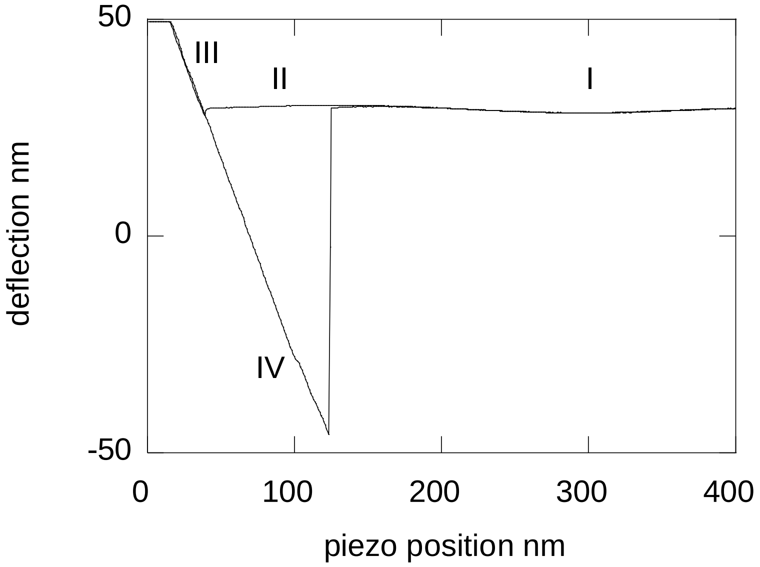

Figure 3.22 Deflection of the cantilever versus piezo position.

To plot the force versus the actual distance between the surfaces the point of contact or zero separation has to be defined. This point is derived from the constant compliance region of the deflection curve plotted versus piezo position. The point where the constant compliance region starts is taken as zero separation point or the point of first contact.

The force between the surface and the probe is usually presented in terms of a force divided by an effective radius of the probe particle R, in Newtons per meter. If the range of interactions and the distance between the surfaces is much less than the effective radius, the force is related to the interaction energy per unit aria U(D), by

$$F(D)/R = 2\pi U(D) \qquad (3.13)$$

This relation is known as Derjaguin approximation. Normalization of the interaction forces and presenting them in F/R units facilitates the comparison of force measurements for particles of different sizes and chemical nature.

3.3.4. Force measurements in air and in water

Force measurements of sulphur particles with silica, cellulose, C18, and apple leaf surfaces were performed both in air and water. In most cases the deflection of the cantilever amounted to about 60-80 nm. Assuming a spring constant of 0.6 nN/nm the forces are between 36 and 48 nN. Results are shown in Figs. 3.23-3.27. The experimental results are presented as the interaction force *versus* distance between the particle and the surface.

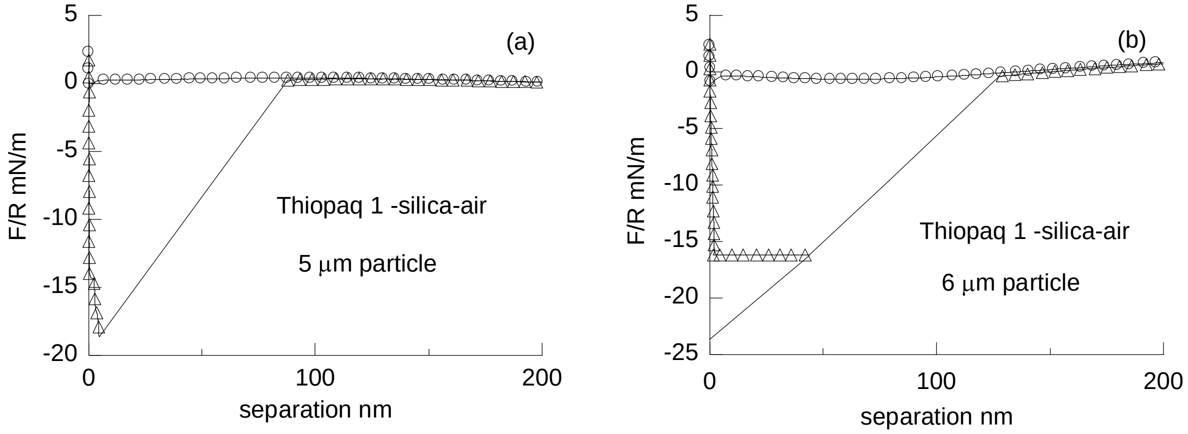

Figure 3.23 AFM force curves for Thiopaq™ sample 1 with a 5 μm and a 6 μm particle on a silica surface in air

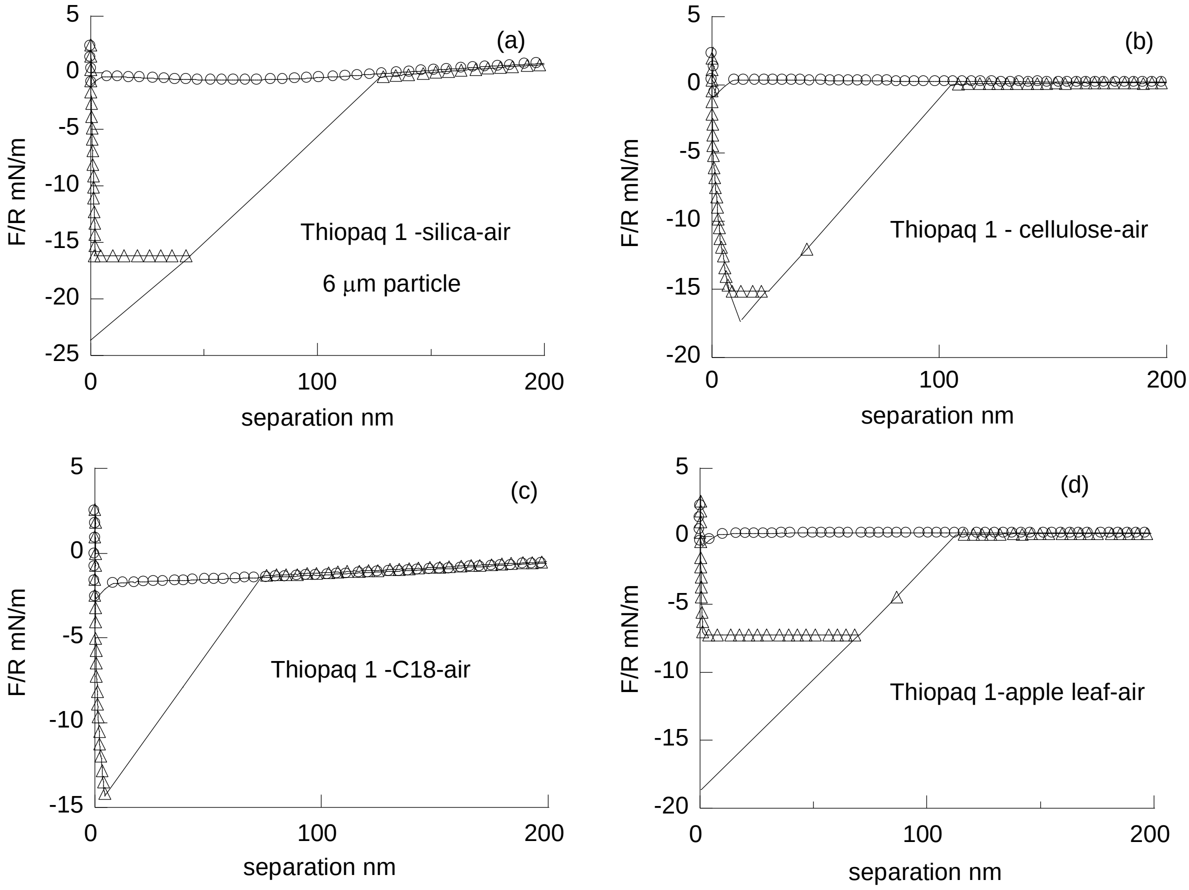

Figure 3.24 AFM force curves for Thiopaq™ sample 1 with a 6 μm particle on a silica surface, a cellulose suface, a C18 surface, and an apple leaf surface in air

The interaction force (F) was normalized by the radius (R) of the particle. Because the particles are rough and not perfectly spherical, the values of F/R are only a rough approximation, but comparison of the different F/R values for the same particle with different surfaces is still very useful.

Firstly, we have compared interaction forces between silica and two Thiopaq 1 particles with different diameters measured in air. The results are shown in figure 3.23. Positive values of F/R correspond to a net repulsion, negative values to a net attraction. The force curves measured upon approach and upon separation are different. At large separations interaction is zero, but at a separation below 10 nm the approach curves show a small attractive interaction. From the retraction curves it follows that there exists a strong contact force which jumps to zero if the contact is lost. The nature of this adhesive force is somewhat uncertain and is higher than the Van der Waals force. If the contact is lost the surfaces rapidly jumped apart to about 100-130 nm distance. In figure 3.23b the retraction force curve shows an artifact in the measurement. The force does not increase above -17 mN/m. The measuring range of the device is limited by the photodiode measuring limit, but the maximum force at zero distance can still be obtained by extrapolation as shown in this figure. The normalized maximum force for the 5 and 6 μm particles are slightly different. For the small particle the adhesion force is about 19 mN/m. For bigger particle a value about 24 mN/m is obtained. The difference must be attributed to the irregular geometry of the particles which makes the normalization procedure somewhat inaccurate. Normalisation by R^2 instead of R seems to be more appropriate. This could be an important aspect for further investigation. To minimize the effect of particle irregularity, a particle once attached to the cantilever was used further in a series of AFM experiments while measuring the interaction force between the probe and different surfaces.

In figure 3.24 the interaction curves between a Thiopaq™ sample 1 particle and surfaces of silica, cellulose, C18, and apple leaf in air are presented. The interaction curves upon approach of a biosulphur particle to these surfaces look very similar. At distances smaller than a few nanometers a small attraction is observed. For all surfaces a strong adhesion/contact force occurs upon separation. It is somewhat surprising that the interaction forces in air are almost identical for the different surfaces and relatively high. It is likely that the roughness of the surfaces, particularly for the apple leaf, is rather different, however it seems that roughness has not a big influence on the contact force. If the origin of the interaction is due to van der Waals' forces, it would mean that the Hamaker constant is similar for the different surfaces. Further investigations are necessary in order to unravel the exact nature of the contact force for these kind of irregularly shaped particles on non-ideal surfaces.

Also large adhesion forces were obtained for the commercial Thiovit® sample (figure 3.25) in air. The formulated Thiovit® particle is covered with lignosulphonate. A characteristic difference is found for the approaching curves with the silica and the cellulose surfaces. For silica already at large distances a significant attractive force is observed and the contact force is much higher than with other systems. A satisfactorily explanation cannot be provided. The distances are too large for Van der Waals' forces to be significant.

Electrostatic attraction is unlikely because in aqueous solution silica, cellulose, and lignosulphonate are negatively charged. Moreover these measurements were performed in air making electrostatic contributions unlikely.

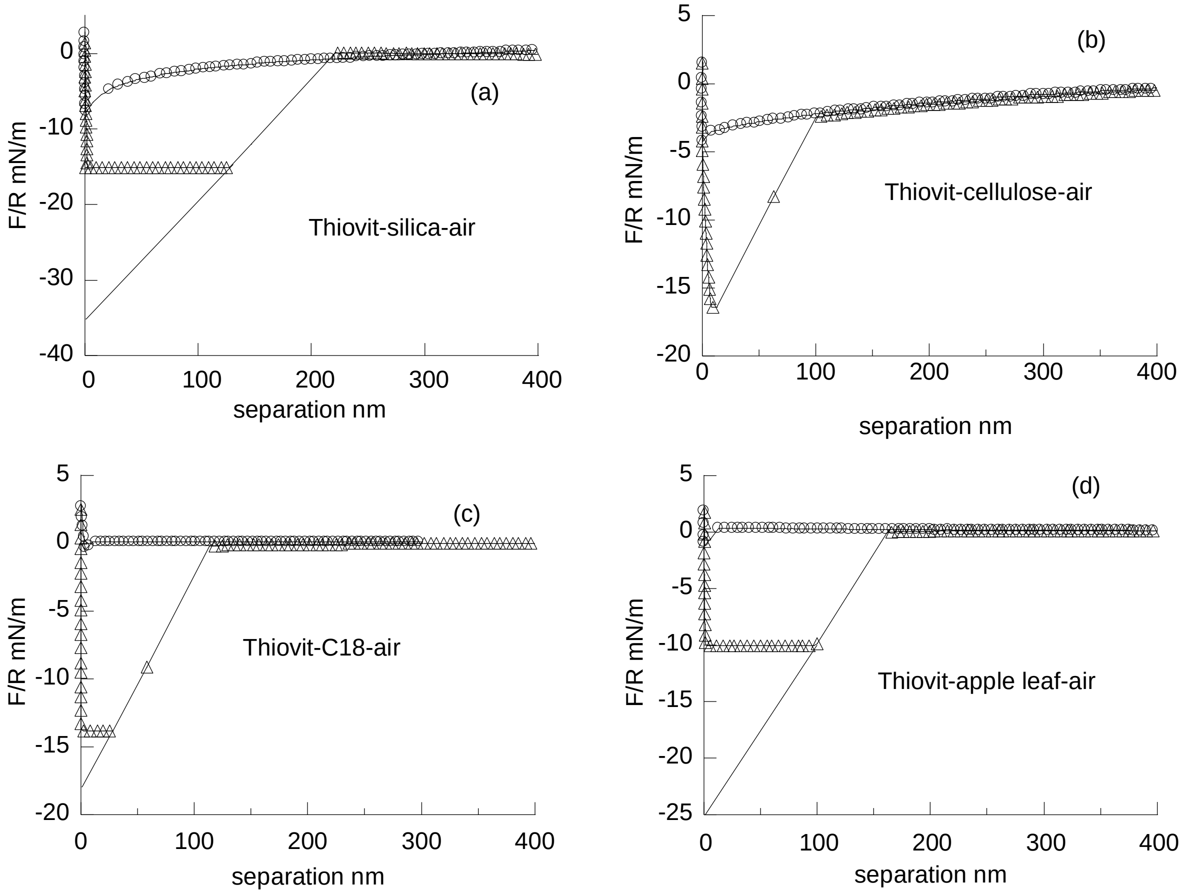

Figure 3.25 AFM force curves for Thiovit$^{®}$ sample 1 with a 6 μm particle on a silica surface, a cellulose suface, a C18 surface and an apple leaf surface in air

In Table 3.8 an overview is presented of the adhesion forces between a Thiopaq and a Thiovit sulphur fungicide particle and the different surfaces in air. For cellulose F/R is almost equal, for C18 and apple leaf the forces with Thiovit® are about 30 % higher, and for silica the adhesion force is even 50 % higher.

Table 3.8. Interaction force in mN/m between a sulphur particle and a flat surface measured in air

Sulphur	silica	Cellulose	C18	apple leaf
Thiopaq™ 1	-23.8	-17.3	-14.5	-18.5
Thiovit$^{®}$	-35.4	-17.2	-18.2	-25.0

Measurements of the contact force between sulphur particles and different surfaces were also performed in water. The results for both Thiopaq™ and Thiovit® particles are shown in figure 3.26 and 3.27, respectively. It appears that the contact force between the sulphur particles and the different surfaces is much smaller in water than in air.

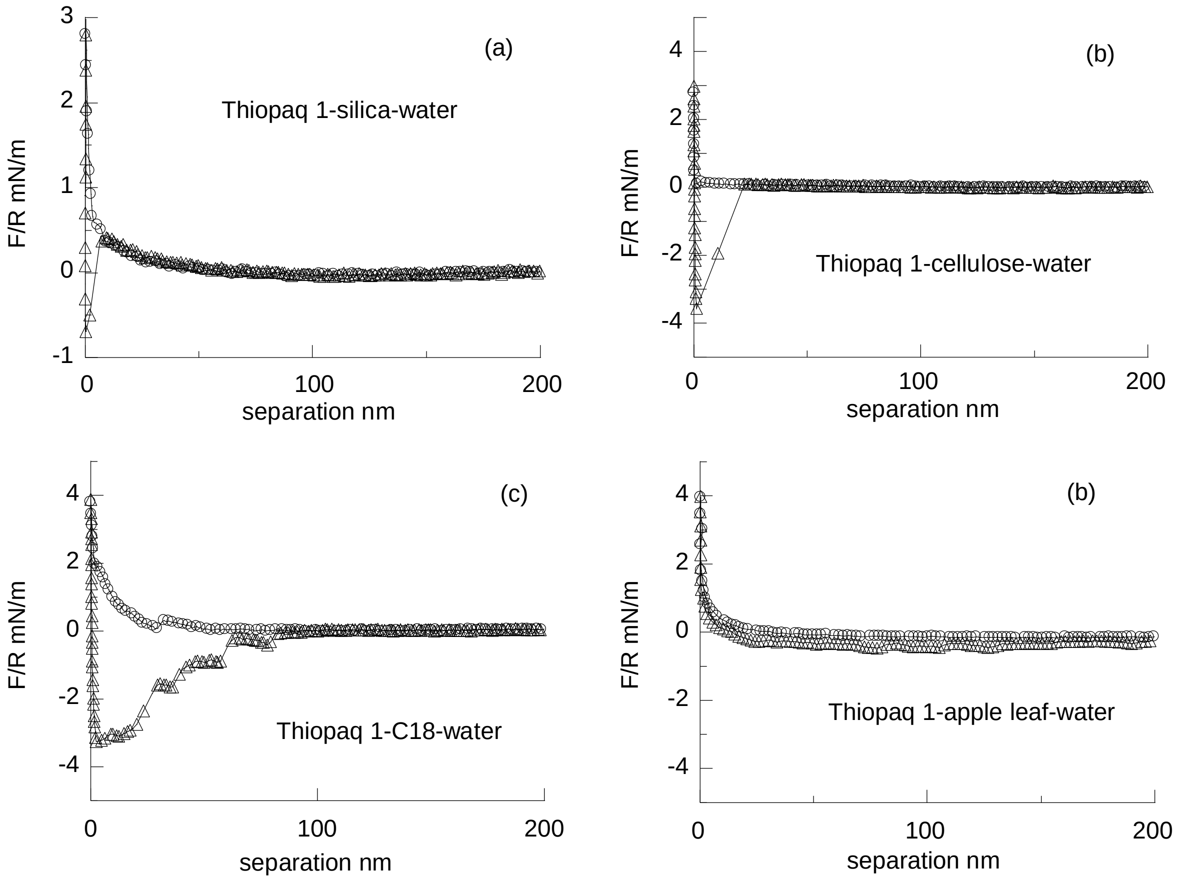

Figure 3.26 AFM force curves for Thiopaq® sample 1 with a 6 μm particle on a silica surface, a cellulose suface, a C18 surface and an apple leaf surface in water

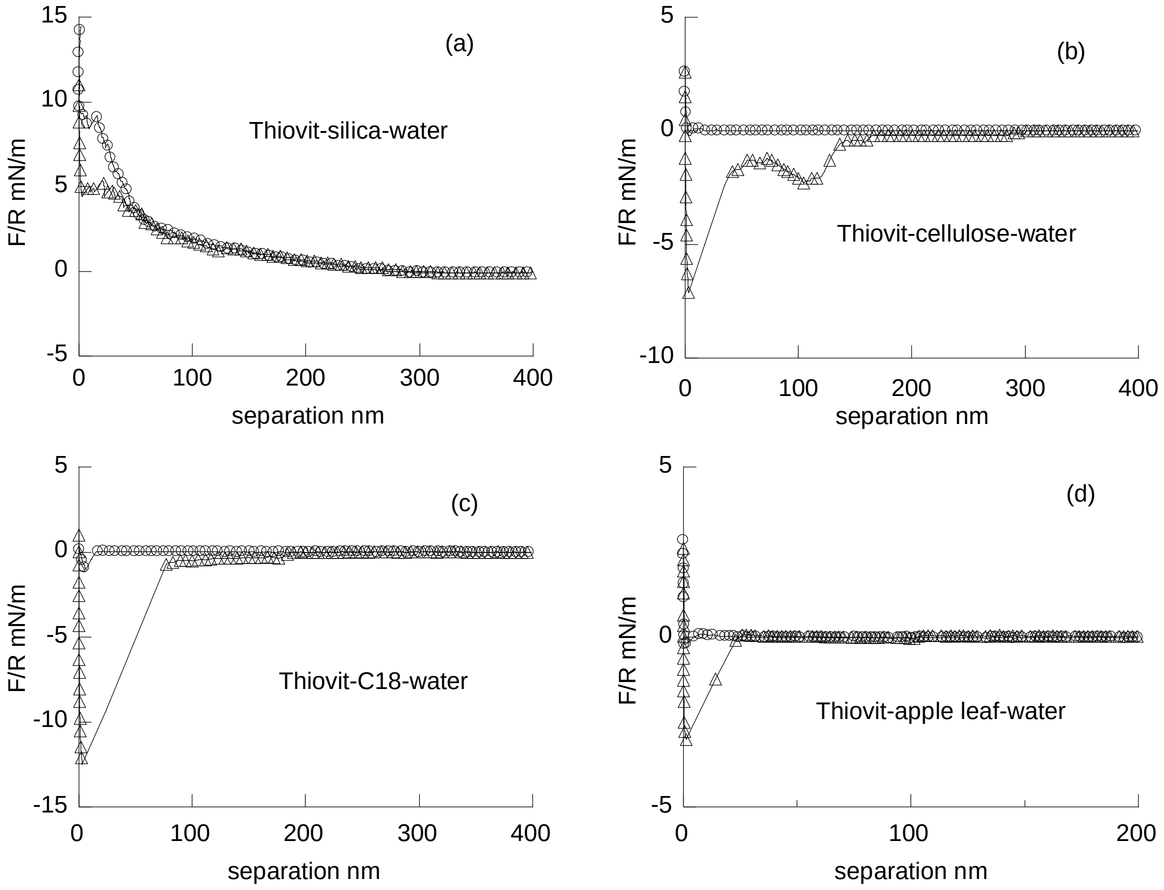

Figure 3.27 AFM force curves for Thiovit™ sample 1 with a 6 μm particle on a silica surface, a cellulose suface, a C18 surface and an apple leaf surface in water

Maximum adhesion (or repulsion) forces upon retraction of the probe from the sample surface in water are summarized in Table 3.9 together with the contact angle. The hydrophilicity of the surfaces decreases in the order: silica>>cellulose>C18. In water, the hydrophilic silica gives only small attractive, or for Thiovit® even a strong repulsive contact force. For the hydrophobic surfaces there exists always an attraction but much lower than in air. Also between the apple leaf and a Thiopaq™ particle a repulsive force was observed. The pronounced differences must be attributed to the presence of additives in the Thiovit® formulation. Most likely, due to the presence of the lignosulphonate the Thiovit surface generates the negative charge in water resulting in electrostatic repulsion between silica and Thiovit probe. For the moderately hydrophilic leaf surface the Thiovit additives changes the contact force from slightly repulsive to slightly attractive.

Table 3.9 Adhesion force (mN/m) between a sulphur particle and a flat surface measured in water. Comparison with contact angles between water and sample surfaces.

Surface	Thiopaq™ 1	silica	cellulose	C18	apple leaf
Thiopaq™		-0.8	-3.8	-3.3	+2.0
Thiovit®		5.0	-7.5	-12.5	-3.2
Contact angle	89.5	0	83	108	70

It must be concluded that force measurements between sulphur fungicide particles and different surfaces give very promising information for the design of an optimal formulation. Nevertheless more detailed research is required for further elaborating this new technique.

3.3.5. Conclusions

The colloidal and surface chemical properties of dried biosulphur particles have been established and the interaction of biosulphur particles with model and leaf surfaces have been determined. Characteristic differences were obtained between the samples dried with different dryers and the native biosulphur sample dried in the laboratory. These differences cannot be attributed to the drying temperature, the origin of the sample, or the type of the dryer. For the dried samples the difference between the specific surface area obtained with nitrogen gas adsorption and adsorption of methylene blue from an aqueous solution was almost absent, whereas for the native sample the area obtained with methylene blue was ten times higher. It seems that the polymeric layer at the sulphur surface behaves different. Also the wetting behavior is surprisingly different. The contact angles for the dried samples are substantially higher than for the native sample and the dried samples are significantly more hydrofobic. On the other hand, the surface charge as obtained from microelectroforesis is similar for the dried and native samples.

Also the particles size distribution seems somewhat dependent on the type of dryer. The dispersion flash dryer gives a single size fraction, whereas with the other dryers fractions with a diameter of about 200 μm are observed. In all cases aggregates were detected. By sonification these aggregates can be destroyed and primary particles with a diameter of 2 μm are obtained (with an additional fraction “doublets” with a diameter of about 4 μm)

With atomic force microscopy images of biosulphur particles have been obtained. These images show the crystalline character of the biosulphur particles and enable an alternative for determining the diameter of the particles. The diameter of the particles correspond satisfactorily with the determination of the particles size distribution.

Interaction forces have been measured between sulphur particles and different surfaces both in air and aqueous environment. It was shown that this method of direct determination of the interaction forces can fruitfully applied to systems with particles with a more complex geometry and to natural leaf surfaces. Most important is, that we can directly monitor the effect of additives on the contact forces. It was shown that the formulated Thiovit particle in water gives an attractive contact force, whereas for the unformulated Thiopaq particles a small repulsion was observed. Thus it can be expected that Thiovit has a better rain-fastness than the unformulated Thiopaq. In future research it should be established, if and which additives are required to obtain an optimal formulation for biosulphur as a fungicide.

3.4. Outlook

This research project has shown the importance of surface and colloid science for developing biosulphur as an effective and environmentally friendly fungicide. Probably, only small of amounts of additives will be necessary to obtain a satisfactorily formulation. In this “seed” project several interesting phenomena were discovered and a new method for measuring interaction forces was further developed for application for irregular particles and with natural leaf surfaces. Therefore there is great potential for continuation of the colloidal and surface chemical research as full PhD-project.
The following aspects deserve further attention.

- *Drying procedure*. The drying procedure with the different commercial dryers leads to a surface characteristic of the biosulphur particles which is surprisingly different from samples dried under lab conditions. Also the effect of the drying procedure on particle distribution is important in further research.
- *Wetting behavior*. Further characterization of the hydrofilicity/hydrofobicity of the sulphur particles is a prerequisite for application of biosulphur as a fungicide.
- *Force measurements.* Force measurements between a colloidal probe and a surface by an atomic force microscope or a dedicated device is a promising new development in colloid and interface science. Application of this method to practical systems and leaf surfaces have not only great value for developing a new biosulphur fungicide but is also important from a fundamental physico-chemical point of view. This technique will be a major subject in a future full project.
- *Model experiments on rain-fastness.* In this project the aspect of rain-fastness has not been studied because of the priority we gave to the force measurements. However, in a full project this subject has to be covered in sufficient detail.
- *Additives for fungicidal biosulphur formulation.* For an optimal application of biosulphur as a fungicide study of the effect of additives will be necessary
- *Rate of evaporation of sulphur fungicide.* Probably, rate of evaporation is the key factor for the efficiency of biosulphur as a fungicide. Comparing rate of evaporation of sulphur samples with different kind of surfaces and different particle size distribution is therefore of big interest
- *Pelletization of biosulphur.* For a better handling of biosulphur fungicide and ease of application, formulating biosulphur as granules which disperse quickly in the tank of a spraying machine will probably be necessary. Both the process of pelletization or granulation as well as the redispersion process contains several interesting colloidal and surface chemical aspects.
- *Application aspects of biosulphur as a fertilizer.* This is another potentially interesting agricultural application of biosulphur. Formulation and rate of oxidation in soil contain relevant physical problems.

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4. BIOSULPHUR AS PESTICIDE

4.1. Introduction

Sulphur is the most important fungicide used in organic production. Moreover, it is the only fungicide in organic apple production against the main disease apple scab, caused by *Venturia inaequalis* in the Netherlands. However, the efficacy of sulphur against apple scab is rather poor under colder conditions. Sulphur is also a major fungicide in conventional culture of grapes, strawberry, many vegetables and several other crops. It has a good efficacy against a wide range of powdery mildew diseases. Sulphur is known also as an acaricide against several species of spider mites and eriophid mites.

Growers have the experience that frequent applications are necessary for sufficient control especially in wet and cool climates. Sulphur is one of the oldest pesticides used in agriculture. Non-the-less, little is known about the rain fastness.

The aim of this part of the project was 1) to evaluate the market for sulphur as a crop protection agent and 2) to test the efficacy of Thiopaq as a fungicide in comparison with standard sulphur (Thiovit Jet). For the latter, three types of experiments are done, i.e. a germination test of conidiospores of *Venturia inaequalis*, an efficacy test against mildew on cucumber and a rain fastness test with apple scab.

4.2. Market survey on the use of sulphur as a crop protection tool

4.2.1. Market study methods used

For this desk study experts were asked about the sulphur market besides the search on the Internet and in literature. These sources were used to estimate the sulphur market and their possibilities in the future.

The world market was described based on a presentation of Mametz (2002). The total consumption of sulphur in the world in the most important crops were given (see table 4.2.3.1). Based on these figures and the world acreage of these crops, the use per hectare was calculated. Cotton, Green Pea and Strawberry were mentioned by Dirkse to be interesting crops concerning sulphur consumption. Estimations about the use per hectare of these crops were based on the information given by Dirkse (see Table 4.3).

There are also estimations made for the sulphur market in the EU. The use of sulphur per hectare in the EU was assumed to be half compared to all other countries, including the developing countries. The same can be said for other crops in the EU. Heijne estimated the rate at which sulphur is used in organic fruit. The database of the FAO delivered the acreage of the crops in the EU.
The estimations about The Netherlands were based on figures of the CBS. They reported data about the use of crop protection products for each crop for each disease. For every combination there were two figures about the volume (in kg) crop protection products used:

- All crop protection products
- All crop protection products without sulphur and without bacterial preparations

Sulphur is especially used against mildew and mites where bacterial preparations are used against caterpillars. So the total use of sulphur and bacterial preparations could be calculated for each crop and disease (mildew, mites, caterpillars etc.) combination. The amount sulphur can be calculated, assuming that bacterial preparations are only used against caterpillars and sulphur is only used against mildew and mites.

4.2.2. Market survey results

Advantages of sulphur
Sulphur is mostly used against powdery mildew and other fungi. In some crops the effectiveness of sulphur against insects is important. Mites are the most important insects against which sulphur is used.
Pests can develop resistance against artificial products for crop protection. The development of resistance is a very important issue in crop protection. Using sulphur does not develop any resistance but sulphur has a lower effectiveness. Using only sulphur is not interesting. It is a very cheap product, therefor using it in combination with synthetic chemicals is interesting. Besides the mentioned advantages it is also friendly for the environment.

4.2.3. The World Market

The world annual market for sulphur using as a crop protection product is between 180 and 200 million Euro. The total amount used is estimated to be around the 200,000 tonnes. Sulphur is used in many crops in different amounts around the world. Sulphur can be used against powdery mildew in crops like: Apple, Grape, Cumin, Beans, Mustard, Mango, Strawberry, Peas and Citrus Fruits. The use as a fungicide is most important. It also has an effect on mites in crops like: Tea, Cotton, Mango, Jowar, Potato, Cucurbits, Roses, Groundnut, Grapevine, Litchi, Jute and Vegetables. The amounts used vary between 1 kg/ha and 30 kg/ha (Mametz, 2002 and www.excelind.com). In table 4.1 is the estimated use of sulphur given in some crops.

Table 4.1. The use of sulphur in some important crops

Crop		Area used in 2002 (x 1000 ha)	Average estimated use per hectare (kg/ha/year)	Total estimated use of sulphur (x 1000 ton/year)
Fruit				
	Grape	7406	16	120
	Citrus	7332	4	30
	Other fruit	35693	0.7	24
	Total fruit	**50431**	**3.5**	**174**
Vegetables				
	Sugar Beet	6041	1	6
	Other vegetables	40923	0.4	16
	Total vegetables	**46964**	**0.5**	**22**
Cereals	**Total cereals**	**658220**	**0.0**	**4**

Source: FAO and Mametz, 2002

The total consumption of sulphur within fruit is about 170,000 ton per year (see table 4.1). The use in vegetables is lower (22,000 ton per year). Grape uses most sulphur per hectare (almost 16 kg per hectare) and is the largest consumer of sulphur in the world (120,000 ton per year). The most important use in this crop is against powdery mildew. In Citrus the protection against mites are interesting. The use of sulphur in cereals only accounts for 2 percent and this is not much when comparing the acreage with for example Sugar Beet. The development of the production area for each crop is a good indication for the development of the sulphur consumption. So presenting the growth in acreage can be interpreted as the growth of sulphur consumption. Table 4.2 gives an overview of the area used in the world for some crops and their share in the consumption world-wide.

Table 4.2. Percentage consumption of sulphur and growth of acreage of some important crops

Crop		% sulphur consumption of total consumption	% growth of acreage between 1998 and 2002
Fruit			
	Grape	60	2,0
	Citrus	15	1,8
	Other fruit	12	7,4
	Total fruit	**87**	**5,7**
Vegetables			
	Sugar Beet	3	-10,9
	Other vegetables	8	23,1
	Total vegetables	**11**	**17,4**
Cereals	**Total cereals**	**2**	**-3,3**

Source: FAO and Mametz, 2002

The consumption of sulphur is biggest in fruit (87% of total consumption). In all categories of fruit the acreage is growing. The area used for Sugar Beet is declining (-10,9%), but the area used for vegetables in general is growing (17,4%). Sulphur is the cheapest miticide in the market (Mametz, 2002). It is not clear if the figures mentioned in table 1 are only about food crops. For this reason cotton is probably not mentioned but this crop has a very big acreage in developing countries (22 million hectares) and is probably a crop where sulphur could be interesting against mites. Although sulphur is cheap, still the amounts used per hectare can be different between crops. Estimations can be made of the use of sulphur per hectare. Table 4.3 gives the result of those estimations. The use of sulphur also differs between countries. For some crops sulphur is interesting in especially developing countries, because it is cheap.

Table 4.3. Estimation of the consumption of sulphur in some crops in developing countries

Crop	Area used (x 1000 ha)	Estimated use of sulphur (kg/ha/year)	Total estimated use of sulphur (x 1000 ton/year)
Cotton	23616	1	24
Green pea	685	20	14
Strawberry	41	10	0.4

Source: FAS, FAO, Mametz (2002), Dirkse (Pers. Com.)

In Table 4.3 are some interesting crops shown concerning sulphur consumption. Grape and Green Pea have a high doses per hectare, but the area for Green Pea is low compared to Grape (see Table 4.1). There is a large area used for cotton but sulphur is probably less important because mites are not the biggest problem in cotton (www.hortnet.co.nz). Cotton in developing countries is interesting because of the price of sulphur and the large area used for cotton. Developing countries have almost 70% of the total cotton production (source: FAO). It is interesting to look at the growth of the acreage of fruit and vegetables in developing countries (Table 4.4). Strawberries are put in the group of fruit.

Table 4.4. The acreage used and the growth in acreage for important crops concerning sulphur consumption.

Group of crops	Crop	Area (x 1.000.000 hectares)		% growth in acreage from 1998-2002	
		Developed	Developing	Developed	Developing
Fruit	Citrus	1.2	6.1	-2.5	2.7
	Grape	5.2	2.2	-0.6	8.7
	Strawberry	0.17	0.04	-6.0	15.2
	Other fruit	5.4	30.1	-0.8	9.1
Vegetables	Green pea	0.3	0.7	-10.0	18.5
	Sugar Beet	4.8	1.2	-11.0	-10.4
	Other vegetables	2.8	37.1	41.3	22.4
Total fruit and vegetables		**19.9**	**77.5**	**0.3**	**14.2**

Source: FAO

Many countries belong to the group of developing countries. For this reason it is not surprising to see that the developing countries use a large area for agriculture. More interesting is to see that the growth in acreage is bigger than in the developed countries (Table 4.4).

The production of grape is done on a bigger area in developed countries than in the developing countries. The growth in acreage of all fruits and vegetables in developing countries was about 14%. Grape is the most important crop in this study. The acreage of Grape has grown only in developing countries and remained constant in developed countries. Crops like strawberry and green pea would also be interesting because of intensive use of sulphur (source: F. Dirkse). The area used for Strawberry is not much, which is also true for Green Pea.

Countries with THIOPAQ installation

The market situation of some countries with a THIOPAQ installation will be described in this section. The countries of interest are: Australia, Canada, China, Egypt and South Africa. Also the southern republics of the Soviet Union are mentioned. This group contains Armenia, Azerbaijan, Georgia, Kazakhstan, Kyrgystan, Tajikistan, Turkmenistan and Uzbekistan.

The crops mentioned in previous paragraph were used to describe the market situation in de ‘THIOPAQ countries’. In previous paragraph grape and fruit in general seemed to have a high need for sulphur. Table 4.5 shows the acreage of fruit in the ‘THIOPAQ countries’.

Table 4.5. Area used for fruit production in ‘THIOPAQ countries’ in 2002 (x 1.000 ha).

	Citrus	Grape	Strawberry	Other Fruit	Fruit total	% area interesting crops* of total area
Australia	31	143	0,7	84	259	68
Canada	-	9	4,7	57	71	20
China	1388	341	0,7	7507	9237	19
Egypt	143	64	2,7	231	441	48
South Africa	69	115	0,7	120	305	61
Southern Republics of former Soviet Union	9	213	2,8	394	619	36

* Interesting fruit concerning sulphur consumption (Citrus, Grape and Strawberry).
Source: FAO

China uses de biggest acreage for growing grape, citrus and fruit in general. Therefore China is probably the biggest market for sulphur of the countries mentioned in Table 4.5. Australia, South Africa and the former Soviet countries have also a large area with Grape. Uzbekistan is very interesting, because about 43% of the total area used for fruit in the former Southern Soviet countries is located in Uzbekistan. More important is their share in the acreage of Grape, which is more than fifty percent of the total acreage in the group of the former Soviet countries. Australia and South Africa have high percentages of Grape and Citrus (see Table 4.5) and are therefore also interesting. The acreage used for Green pea, Sugar Beet and vegetables in general are shown in Table 4.6.

Table 4.6. Area used for vegetable production in 'THIOPAQ countries' in 2002 (x 1.000 ha).

	Green pea	Sugar Beet	Other vegetables	Vegetables total	% area interesting crops of total area*
Australia	5	-	80	85	6
Canada	16	10	87	114	24
China	211	424	19737	20372	3
Egypt	24	65	467	556	16
South Africa	3	-	128	131	2
Southern Republics of former Soviet Union	3	47	541	591	9

* Interesting vegetables concerning sulphur consumption are: Green Pea and Sugar Beet.
Source: FAO

Also concerning vegetables China represents the biggest market for sulphur (see table 4.6). Egypt is also interesting because it has a big area for Sugar Beet and Green Pea. Canada has a high percentage of land used for Sugar Beet and Green Pea (see table 4.6). Still when making a final analysis the vegetable production is less important than fruit for estimating the sulphur consumption.

Cotton is also one of the interesting crops mentioned in the previous paragraph. The top seven producers of Cotton are China, the United States, India, Pakistan, Uzbekistan, Turkey and Brazil (www.agjournal.com). Concerning the 'THIOPAQ countries' China and Uzbekistan are the main producers of Cotton. Still for cotton it is not easy to estimate the use of sulphur for each country.

Finally the sulphur consumption is estimated based on the use per hectare described in the previous paragraph. In Appendix 1 and 2 the estimated use of sulphur per hectare for each country is given. The total estimated consumption is displayed in Table 4.7.

Table 4.7. Total estimated consumption of sulphur in fruit en vegetables for conventional crop protection.

Country	Expected sulphur market (x 1.000 kg)
Australia	1644
Canada	299
China	33459
Egypt	2698
South Africa	2367
Southern Republics of former Soviet Union	4291

Source: FAO, Mametz (2002) and Dirkse (Pers. Com.)

Of all the countries described in Table 4.7, China has the biggest market for sulphur. The southern republics of the former Soviet Union may also use a lot of sulphur. When looking more to the countries representing this group, Uzbekistan seems to be the most interesting. This country was also mentioned to have a significant Cotton production, but is also responsible for almost half of the estimated sulphur consumption in fruit en vegetables.
We have discussed the sulphur consumption in conventionally grown fruit and vegetables. It is not easy to find figures about acreage of organic grown Grape for example.

The acreage of all organic production was described in Yussefi and Willer, 2003. This acreage includes dairy products, meat, fruit, vegetables etc. This study shows that especially Australia is a very big producer of organic products. Australia has 10.5 million organic hectares and is therefor in the world by far the biggest producer of organic products. Canada and China have respectively 0.4 and 0.3 million hectares for organic production. All countries except Australia use less then one percent of the total agricultural area for organic production.

The European market

The total Sulphur market in the EU is between 40 and 45 million Euro (Pers. Com. Raymond Mametz). It is not clear how the amount is divided over de crops in Europe or the EU. The EU is one of the biggest growers of Grape. About 45% percent of the total world acreage are situated in the EU. The second largest consuming crop is probably a fruit crop but there are no figures found about the use of sulphur in the EU of Europe. Strawberry in the south of Europe has also a high consumption of sulphur per hectare (source: Dirkse)**.** The use of sulphur on Grape per hectare is probably lower in the EU than in the world and especially lower than in the developing countries. In Table 4.5 are the areas shown of Grape, fruit (conventional and organic) and Strawberry in the EU. Vegetables are not shown because they are less important (see Table 4.1), especially for de developed countries where grape is expected to be a large consumer.

Table 4.8. Use of sulphur in the EU in different crops.

Crop	Area used in 2002 (x 1000 hectares)	Estimated use of sulphur (kg/ha/year)	Total estimated use of sulphur (x 1000 ton/year)
Grape (wine and table)	3323	10	33
Fruit* (conventional)	1988	1	2
Fruit exc. melons exc. grape (organic)	68**	30	2
Strawberry***	27	4	0.1

Source: FAO and Mametz, 2002
*Exc. grape, melons and production of strawberry in the south of the EU.
**Estimation based on Weibel (2002) and information from FIBL
***Calculation for the South of the EU

There are about 3,3 million hectares of Grape in the EU. With an estimated average use of 10 kg per hectare, this market uses 33,000 ton sulphur per year. The average use per hectare was 16 kg (see Table 4.1), but it is assumed that developing countries would use relatively more sulphur than developed countries. Therefor the use was estimated to be 10 kg per hectare. The use of sulphur in strawberry is most important in the south of Europe, but the area isn't much. Poland is a very big producer of strawberries (source: FAO), but it is not clear how much sulphur is used in Poland. The use can differ between countries in Europe. The climate has influence on the effectiveness. The higher temperatures in the south of Europe are probably more useful than the temperatures in the north. Regulations about the allowed use of crop protection products can also differ. This is also true between countries within the EU.

In the EU the environmental care is an important issue. The EU is stimulating the organic sector. Organic agriculture is an important market for sulphur because there are not many ways of crop protection, which don't harm the environment too much. The use of sulphur in organic production is vital, because it is considered relatively environment friendly. A growing use of organic production will result in a simultaneously growing demand for sulphur. The rapid growth of organic production in the EU resulted in large area's used for organic production in some countries (see Table 4.9).

Table 4.9. Organic area used in Europe in 2001.

Country	# hectares organic	% organic land of total agricultural area
Austria	285500	11.3
France	419750	1.4
Germany	632165	3.7
Greece	31118	0.6
Italy	1230000	7.94
The Netherlands	38000	1.94
EU	**4442876**	**3.24**
EU Accession	555139	0.28

Source: FIBL

In Table 4.9 we can see that the area used for organic production in general (dairy, fruit, meat, vegetables etc.) is about 3 percent in the EU. The area used for organic agriculture is different between countries. Greece has the lowest percentage in the EU and has a similar area as The Netherlands. More recent figures show a percentage of 2.25 to be organic in The Netherlands (Anonymous A, 2003). Austria has the highest percentage (11.3%), but Italy is obviously the biggest organic producer (about 1 million hectares). The organic area in the EU has grown for many years, but in some countries the growth seemed to have stopped. The EU is making policy to stimulate organic agriculture. It is not clear which potential acreage is to be expected. This could increase the demand for sulphur. In the Netherlands the government wants 10% of the agricultural production to be organic in the year 2010 (Yussefi and Willer, 2003). The area used for organic agriculture in The Netherlands grew 3 percent last year (Anonymous A, 2003). The organic production in the EU accession countries is growing every year, but the average is still lower than the smallest producer Greece (see Table 4.9). Therefor the accession countries have a big potential growth for organic agriculture compared to the EU.

The Dutch Market

The Dutch situation is more or less different compared to other countries in Europe. The Dutch government has a different policy in regulation of crop protection products. Fewer chemicals for crop protection are allowed to use in The Netherlands compared to other EU countries. There would be fewer products left if regulation in the EU would change towards the Dutch standards. Sulphur would still be allowed, because it is relatively save for the environment and consumers (Mametz, 2002). In that case sulphur would be more interesting. Therefor it is interesting to look at the use of sulphur in The Netherlands to see what effects can be expected.

There are no exact figures found but there are some good indications about the use of Sulphur in The Netherlands. The total use as a protection tool is estimated around the 100 ton (Pers. Com. Ton Besseling). In Table 4.10 the use of Sulphur is estimated based on figures from CBS.

Table 4.10. Estimated use of Sulphur in The Netherlands.

Year	Total use of sulphur (ton)
1995	63
1998	105
2000	85

Source: CBS

In 2000 about 85 tonnes of sulphur was used, but there is no real trend to be seen over the years (table 4.10). It only gives the idea of how much sulphur is used. Nowadays the use is probably bigger, because regulation has changed a lot about the use of products for crop protection since 2000. Some important products are forbidden in 2003 and this process is still continuing. There are some crops where a lot of sulphur is used. The figures of these crops are displayed in table 4.11.

Table 4.11. The use of sulphur in the most interesting crops (integrated production).

Crop	Estimated use of sulphur in 2000 (kg)
Apple	21728
Hedging-plant material & Forest plant stock	5044
Rose bushes	4963
Tomatoes	17659
Sweet pepper	6443
Roses	22637
Gerbera	3449
Total	**81923**

Source: CBS

Table 4.11 gives us also a total amount of sulphur which is almost the total amount given in Table 4.10 (85 ton). About 90% of the total amount is consumed in the crops mentioned in Table 4.8. Apples, Tomatoes and Roses are the most important crops according to these figures. Pear is not shown because it is a less important crop considering the use of sulphur as crop protection tool. Sulphur was not allowed as crop protection method in pear, but was allowed as fertiliser. Farmers used the fertiliser to do the crop protection, because it was almost six times cheaper than alternative products. Therefor the use in Pear is probably higher than the figures of the CBS. Sulphur made for crop protection, for example Thiovit® Jet, will cost about €2,5 per kg (source: AGE Rijnvallei) in the Netherlands and is cheaper than the fertiliser. For vegetables and fruits between 3 and 6 kg of Thiovit® Jet per hectare is advised to use against powdery mildew per application www.syngenta.be). About 80-100 kg/ha/year is no exception for organic apple production. Tomato seemed to be an important crop in The Netherlands concerning sulphur. So it is possible that the developing countries would also use sulphur in tomato. It is not certain if the same holds for other developed countries, because in other developed countries more chemicals are allowed than in The Netherlands. There is a big area used for tomato in the world (almost 4 million hectares). Roses are also important but are grown on a small scale when compared with food crops.

4.2.4. Market survey discussion

It is not clear if the 200,000 tonnes sulphur (see 4.2.3) is only consumed in food crops or also in non-food crops like cotton. So the world market could be larger than expected. Nevertheless in the Dutch market ornamentals play an important role in de consumption of sulphur. There was a very little consumption within vegetables. More use was expected, but sulphur is probably just not interesting enough for the Dutch climate. This could be interesting when the production of Tomato under glass increases in Europe. The temperatures in glasshouses are in general good for an effective result of sulphur.

More and more products can not be used in the EU because of regulation, for this the demand for sulphur will grow. However crop protection companies will develop environmental friendly products in the future.

It was also mentioned that developing countries would play an important role in the demand for sulphur because it is a cheap solution. On the other hand these countries may develop and have money for more specific fungicides and miticides.

4.2.5. Market survey conclusions

The demand for sulphur will grow. This conclusion is based on a few trends:

- The growing acreage and production in developing countries
- The growing interest for the environment in the EU
- The growing acreage of fruit in general and grape in particular

Sulphur is not expensive and is a relatively cheap solution for the poor farmers in developing countries. Even in these countries the acreage of Grape is growing. In the EU the acreage of Grape remains on a constant level, but regulations about the allowed use of some chemicals could increase the use of sulphur. Besides the conventional agriculture, there is also the growing demand from the organic agriculture were less products are allowed. It is safe to say that increase in acreage for organic production will result in a simultaneously increase in sulphur consumption. This is especially true for fruit, which is in conventional use the biggest consumer of sulphur.

Of the ‘THIOPAQ countries’ China is the most interesting one. For conventionally grown crops Egypt, South Africa and Uzbekistan are interesting. Australia can be interesting because of their organic production.

4.2.6. Market survey literature

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Yussefi, M. and Willer, H., 2003. The World of Organic Agriculture – Statistics and Future Prospects. Tholey-Theley, International Federation of Organic Agriculture Movements.

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Mametz, R., 2002. Markets for sulphur as a fungicide. Presentation at The sulphur Institute's eight-biennial international symposium 'Sulphur Markets-Today and Tomorrow', Amsterdam The Netherlands.

Sources:

F. Dirkse (Modify b.v.)
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Ton Besseling (Syngenta)
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Appendix 4.2.1: Estimated sulphur consumption in fruit production in 'THIOPAQ countries' (kg/ha/year).

Country	Citrus	Grape	Strawberry	Other Fruit
Australia	2	10	4	1
Canada	2	10	4	1
China	4	16	10	1
Egypt	4	16	10	1
South Africa	4	16	10	1
Southern Republics of former Soviet Union	4	16	10	1

Source: FAO November 2002 and Mametz, 2002

Appendix 4.2.2: Estimated sulphur consumption in vegetable production in 'THIOPAQ countries' (kg/ha/year).

Country	Green pea	Sugar Beet	Other vegetables
Australia	5	1	0,5
Canada	5	1	0,5
China	20	2	0,5
Egypt	20	2	0,5
South Africa	20	2	0,5
Southern Republics of former Soviet Union	20	2	0,5

Source: FAO November 2002 and Mametz, 2002

4.3. Efficacy of Thiopaq™ biosulphur as pesticide. Spore germination test

Different doses of Thiopaq sulphur and Thiovit-Jet (80 % sulphur, Syngenta) were mixed with water agar. The agar was poured in Petri-dishes. Conidiospores of *Venturia inaequalis,* the causal fungus of apple scab, were collected from a have infested orchard. The spores were spread over the surface of the agar plates. Agar plates were incubated at ambient room temperatures varying between 16 and 21 °C. Germination of spores was counted under a microscope at 48 hours after start of incubation. A spore is counted as germinated when the germ tube is longer than the length of the spore. Treatments were untreated, Thiopaq at 0.01, 0.1, 1 and 10 % and Thiovit-Jet at 0.4 and 4 %. There were three replicates (Petri-dishes) per treatment.

4.3.1. Results of the spore germination test

In figure 4.1 the percentage germination is shown. It is demonstrated that Thiopaq sulphur is equally effective as the standard fungicide Thiovit-Jet. A dose of four percent is necessary to completely prevent the germination of conidiospores of Venturia inaequalis for both products in this severe test.

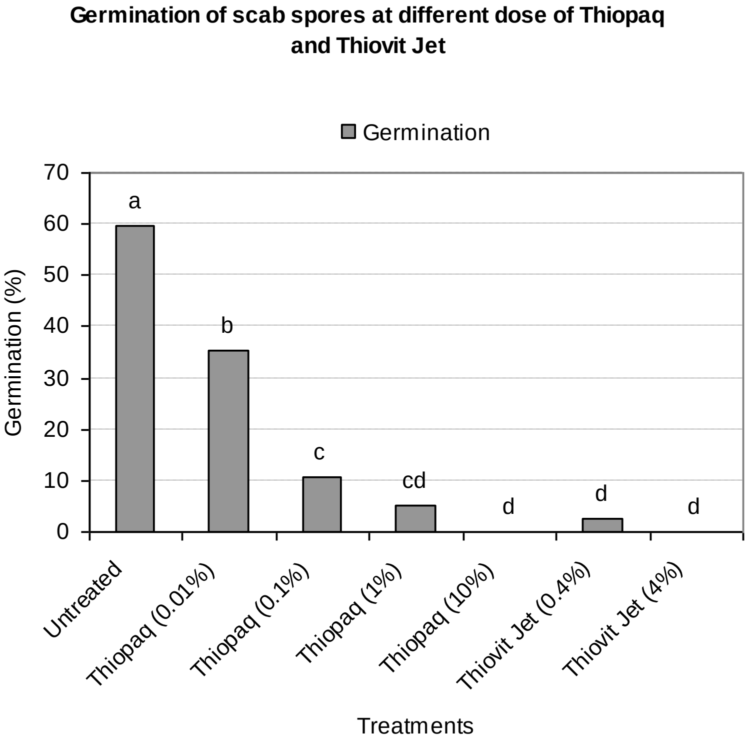

Figure 4.1 The percentage of germination of conidiospores of Venturia inaequalis at different doses of Thiopaq sulphur and Thiovit.

4.4. Thiopaq efficacy against mildew of cucumber

4.4.1. Materials and methods

Two experiments were done to determine the efficacy of Thiopaq Sulphur against mildew in cucumber caused by *Sphaerotheca fusca* in comparison with Thiovit Jet. For that purpose, young, mildew-sensitive plants of cultivar Euphoria were bought from a commercial grower. Individually potted plants were considered as experimental units. The experiment were done according to a completely randomised design with 6 replicates.

Plants were sprayed with the different sulphur solutions. A concentration range of Thiopaq sulphur and Thiovit Jet (80 % sulphur, Syngenta) was prepared ranging from 0.1 to 4.8 and 0.04 to 1.5 g/l respectively for experiment 1 and 2. The surfactant Agral LN was added to the spray solutions at the rate of 1 ml/l in experiment 2 only. After drying, plants were inoculated with powdery mildew. Plants infected by powdery mildew were obtained from colleagues at Applied Plant Research for greenhouse crops at Naaldwijk, the Netherlands. Inoculum solution in water was prepared by gently brushing spores into the water. Then, the water was filtered through cheese cloth to remove larger mycelial parts and hairs from the leaves. The number of spores of the inoculum solution was counted with a haemocytometer. Inoculated plants were placed in a climate room at 20 °C and ambient relative air humidity ranging from 70 to 95 %.

A mildew index was calculated to evaluate the results of the experiments. Infection of individual leaves was estimated in five classes: class 0: no mildew; class 1: a few spots of mildew, 0 – 5 % of leaf infected; class 2: 5 – 25 % of the leaf infected; class 3: 25 – 75 % of the leaf infected; class 4: > 75 % of the leaf infected. The index was calculated as the mathematical average of the classes according to the formula: index = $(I_1 + I_2 + \ldots + I_n)/n$, where I_1 to I_n = the class number of individual leaves and n = the number of leaves per plant. Genstat 6th Edition was used for a non-linear regression analysis.

4.4.2. Results and discussion on the efficacy against mildew of cucumber

The efficacy of both types of sulphur decreased with a decreasing dose as expected (figure 4.2 and table 4.3). Therefore it is concluded that both types of sulphur are effective against mildew of cucumber. Thiopaq sulphur appeared to be more effective than Thiovit-Jet in the first experiment (figure 4.2). Thiopaq was less effective than Thiovit-Jet in the second experiment in which a surfactant was added to the spray liquid (figure 4.3).

Table 4.12. The effect of the dose of sulphur on mildew on cucumber expressed as a Mildew index; high index data indicates a heavy infestation.

Experiment 1				Experiment 2			
treatment	product and dose (g/l)		mildew index	treatment	product and	dose (g/l)	mildew index
1	Thiovit	0.0	3.625	1	no surfactant	0.0	-
2	Thiovit	0.08	1.833	2	with surfactant (+)	0.0	3.167
3	Thiovit	0.2	0.750	3	Thiovit (+)	0.05	1.667
4	Thiovit	0.4	0.500	4	Thiovit (+)	0.1	2.083
5	Thiovit	0.6	0.333	5	Thiovit (+)	0.175	1.917
6	Thiovit	1.2	0	6	Thiovit (+)	0.25	1.667
7	Thiovit	2.4	0	7	Thiovit (+)	0.5	0.750
8	Thiovit	4.8	0	8	Thiovit (+)	0.75	0.917
9	Thiopaq	0.0	3.625	9	Thiovit (+)	1.5	3.167
10	Thiopaq	0.08	0.917	10	Thiopaq (+)	0.04	2.500
11	Thiopaq	0.2	0	11	Thiopaq (+)	0.08	2.667
12	Thiopaq	0.4	0	12	Thiopaq (+)	0.14	2.167
13	Thiopaq	0.6	0	13	Thiopaq (+)	0.2	1.750
14	Thiopaq	1.2	0	14	Thiopaq (+)	0.4	1.333
15	Thiopaq	2.4	0	15	Thiopaq (+)	0.6	1.167
16	Thiopaq	4.8	0	16	Thiopaq (+)	1.2	0.750

It was anticipated that a surfactant should improve the distribution of spray liquid over the leaves, resulting in a better efficacy. However, the efficacy of both sulphur types was about half of that from the first experiment in which no surfactant was added. The results indicate that there might be an interaction between sulphur type and surfactant. Consequently, the type of sulphur might be different in their rain fastness. This matter is not yet understood and needs further investigations.

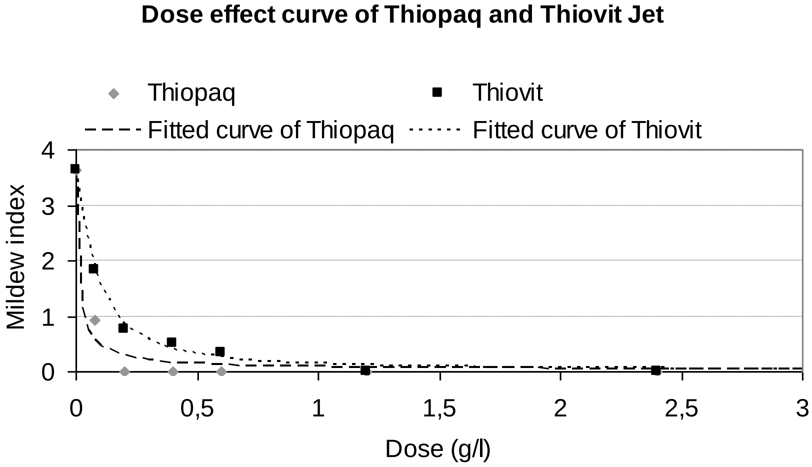

Figure 4.2. The dose effect curve of Thiopaq and Thiovit Jet without additives

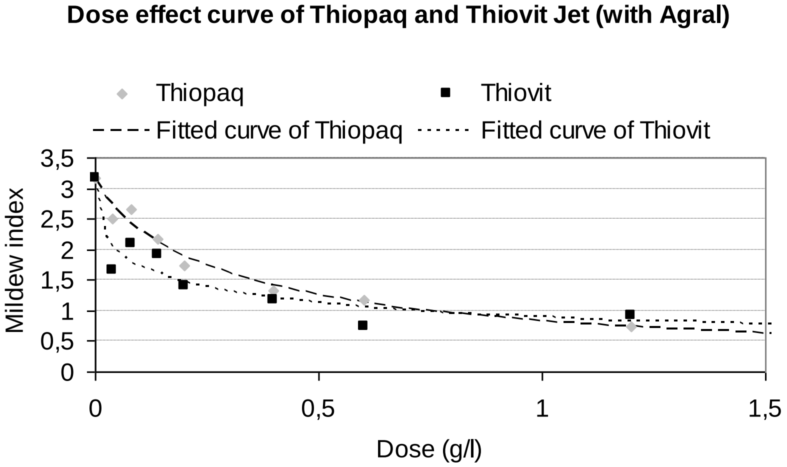

Figure 4.3. The dose effect curve of Thiopaq and Thiovit Jet with the surfactant Agral (0.1 %) added.

4.5. Rain fastness and efficacy against apple scab

4.5.1. Summary of the trial set-up

Potted apple trees were treated with sulphur. Different quantities of artificial rain ware applied to the apple trees. Subsequently, a number of the leaves were taken from the apple trees for analysis of the remaining sulphur residue. Then, the apple trees with remaining leaves were artificial inoculated with apple scab and placed in a climate chamber. Apple scab symptoms were counted about two weeks after inoculation.

4.5.2. Methods rain fastness and efficacy against apple scab

Potted plants of M. 9 rootstocks of apple were used for the rain fastness experiment. Two plants were potted per one 10-liter pot. Plants were at least two years old. Pots were placed in a cold-storage chamber at 4 °C from the winter onwards to start of the experiment. Pots with plants were taken out of the cold-storage chamber five weeks before start of the experiment at 11 August 2003 and placed under roofing with side walls made of netting. Hence, the pots with plants stayed dry during natural rain. Plants were fertilised, watered with drip irrigation and not treated with pesticides.

Artificial rain was produced with an apparatus manufactured by Hol & Zn., Meteren, the Netherlands. It consists of a 1000 l tank mounted on four wheels. Five Teejet DG 8003 flat fan nozzles on a horizontal boom were operated at low pressure by a pomp with backwash. The spray boom was 2.15 m above soil surface. Potted plants were place on square meter boxes 0.65 m above soil surface during application of artificial rain.

Treatments rain fastness with apple scab

Thiopaq and as a standard, Thiovit Jet (80 % sulphur, Syngenta) were applied at 16 September 2003 on plants except non-treated control plants at 4.8 and 6 kg/ha respectively with a motor knap-sack sprayer with a fine nozzle and air support at 200 l/ha. Artificial rain treatments are shown in Table 4.13.

Table 4.13 Treatments

treatment Thiovit Jet		treatment Thiopaq sulphur	
1	0 mm rain, non-treated with Thiovit	9	0 mm rain, non-treated with Thiopaq
2	0 mm	10	0 mm
3	5 mm	11	5 mm
4	10 mm	12	10 mm
5	15 mm	13	15 mm
6	20 mm	14	20 mm
7	30 mm	15	30 mm
8	40 mm	16	40 mm

Plants with 0 mm rain were placed immediately in a cold-storage room at 4 °C. Potted plants were placed under the spray boom in two rows according to a randomised block design with four replicates two hours after drying of the sulphur treatments. One pot with two plants was the experimental unit.

One legally stamped KNMI rain gauge was placed half way the row of plants to measure the quantity of rain. Beside the rain gauge, plastic trays 27.5 x 36.5 x 17.5 cm, of approximately 0.1 m^2, were used to collect rain to check the quantity on six other places in the row of pots. A stopwatch was used to determine the point in time of approximately the required amount of rain, i.e. 5 mm rain for the first time. Raining was stopped at those moments and plants of that treatment were removed. The actual quantity of rain was measured and raining started again for the remaining treatments. The removed plants were placed elsewhere under the roofing till they were dry. After drying, leaf samples were taken for residue analysis. Then, the plants were translocated to the cold-storage chamber at 4 °C. This procedure was repeated after each quantity of rain.

Inoculation rain fastness with apple scab

Two small scale tests were done to check inoculation procedure under cold conditions. Firstly, 0.1 ml of 0, 4 and 6 g/l Thiovit Jet solution was spread over the surface of water agar in Petri-dishes. After drying, 0.1 ml of a *Venturia inaequalis* condiospore solution of 3 x 10^5 spores/ml was spread over the surface of the water agar. Percentage spore germination was counted after placing the Petri-dishes 48 hours at 8 °C in a climate chamber at 26 June 2003.

Secondly, three pots with M.9 apple plants were treated with 0.6 % Thiovit Jet solution and three others were left untreated. After drying, plants were inoculated with 1,275 x 10^5 spores/ml and placed in a climate room at 8 °C and 100 % relative air humidity at 11 June 2003. Plants were transferred to the outdoor roofing with wall of netting after 48 hours. Symptoms were counted three weeks after inoculation. A scab infection index was calculated by classifying leaves with 1 – 5 lesions in class 1, 6 – 10 lesions in class 2, 11 – 15 leaves in class 3 and over 15 lesions in class 4.

Conidiospores of *Venturia inaequalis* were collected by washing with tap water and gentle brushing of infected leaves from an untreated orchard. The spore numbers were counted with a haemocytometer at 7.2 x 10^4 spores/ml. In total 5 l spore suspension was used to inoculate all plants. For that purpose, plants were taken out of the cold-storage chamber at 18 September 2004, inoculated and randomly placed in the climate room at 8 °C and 100 % relative air humidity. Temperature and humidity were checked by Hobo data-loggers (manufacturer: Mulder Hardenberg B.V., the Netherlands). Plants remained under these conditions for 48 hours. Subsequently, the plants were transferred to the outdoor roofing with wall of netting for symptom expression. The number of shoots, total number of leaves, leaves with symptoms and the number of scab lesions were counted on 9 October 2003, three weeks after inoculation.

Sulphur analysis of residue on apple leaves

Two recovery tests were done to check the sulphur recovery from apple leaves. Solutions of 0, 0.5, 1, 2.5, 5 and 10 g/l Thiovit Jet were prepared. A droplet of one ml of each solution was placed on an apple leaf in five replicates. After drying, leaves were stored at 7 °C in a refrigerator till analysis. Each leaf was shaken for two hours in 60 ml of acetone in a Gerhardt apparatus at position 7.5. The found quantities were compared to verifying curves from both Thiovit Jet and pure sulphur (Riedel de Häen). Thiovit Jet solutions of 0, 0.6, 2.1, 4.2, 8.4 and 16.8 g/l were used in a second recovery test. Analysis of sulphur was performed with High Pressure Liquid Chromatography apparatus (HPLC). Sulphur peaks appeared after 9 minutes.

Fifteen leaves per pot were used for sulphur residue analysis from the rain fastness experiment. Leaves were shaken for two hours in 90 ml of acetone in a Gerhardt apparatus at position 5. The acetone solution was stored at 7 °C in a refrigerator till HPLC analysis. The surface of each leaf was measured with an Eijkelkamp surface measuring apparatus.

Statistical analysis

Genstat 6th Edition was used for statistical analysis. Non-linear regression analysis was used for the bio-assay results and linear regression analysis was used for sulphur residues.

4.5.3. Results of rain fastness and efficacy against apple scab

Germination and inoculation tests

The germination of conidiospores of *Venturia inaequalis* was strongly inhibited by sulphur at 8 °C. The percentage inhibition was 93.4 and 92.6 for respectively 4 and 6 g/l Thiovit Jet with respect to the untreated control (figure 4.4). There were no significant differences between the two doses of Thiovit Jet.

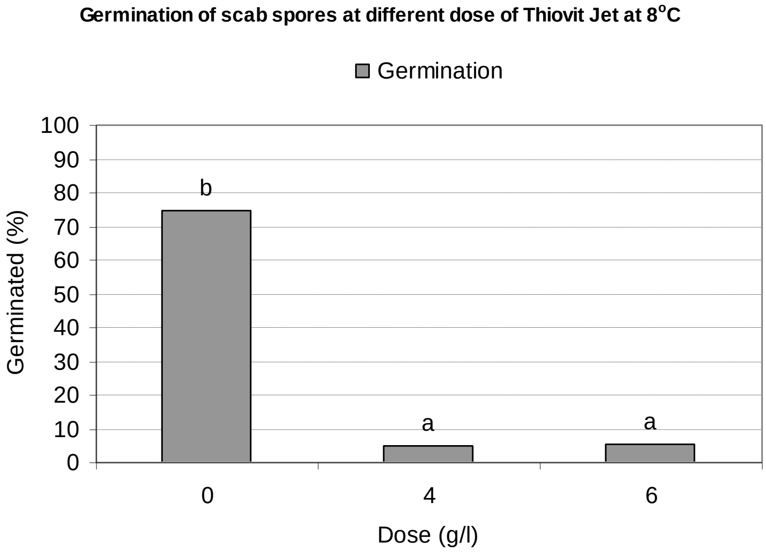

Figure 4.4. Percentage germination of conidiospores of *Venturia inaequalis* after treatment with Thiovit Jet at 0, 4 and 6 g/l incubated at 8 °C on water agar plates.

The incidence of apple scab defined as the number of leaves per shoot with symptoms was reduced by 87 % when plants were treated with 6 g/l Thiovit Jet at 8 °C compared to the untreated control (figure 4.5a). The severity of apple scab defined as the number of lesions per leaf and expressed as a scab index was reduced by 95 % by the treatment with 6 g/l Thiovit Jet at 8 °C compared to the untreated control (figure 4.5b). However, the sulphur treatment did not control the disease completely under these conditions.

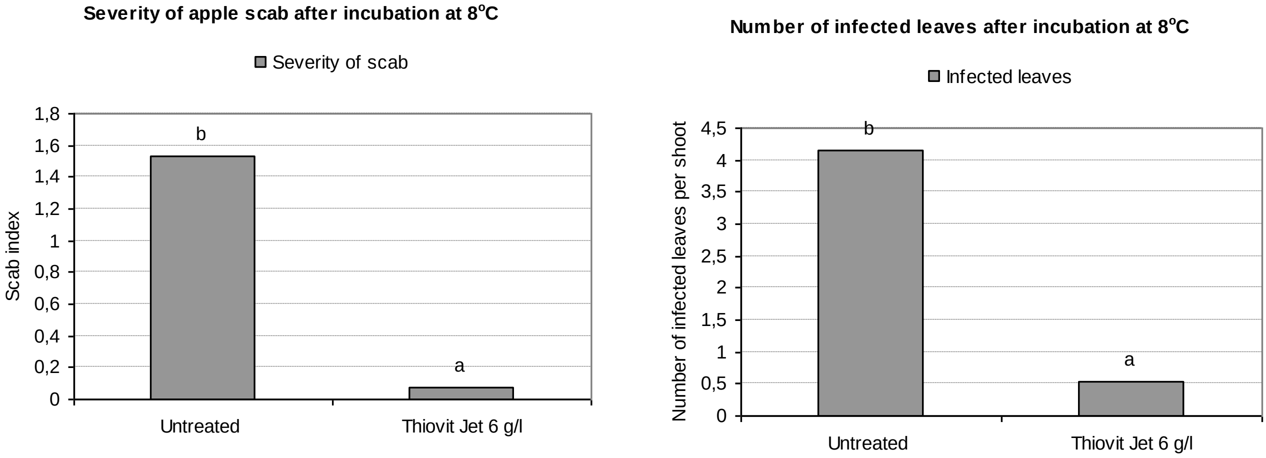

Figure 4.5a and 4.5b. Incidence (a) and severity (b) of apple scab when treated with and without 6 g/l Thiovit Jet incubated at 8 °C.

Sulphur recovery tests

Two times recovery tests were made, in June and August. The sulphur recovery tests showed a slightly variable but acceptable recovery (Table 4.14 and 4.15)

Table 4.14. The percentage sulphur after analysis method June.

Concentration (mg/l)	Volume (l) on Leaves	Thiovit (mg) on leaf	Volume (l) acetone	Calculated concentration (mg/l) in sample	Analysed concentration (mg/l)	% Recovery
0	0,001	0	0,06	0	0	-
500	0,001	0,5	0,06	8,3	8,19	98,7
1000	0,001	1	0,06	16,7	17,56	105,2
2500	0,001	2,5	0,06	41,7	42,31	101,5
5000	0,001	5	0,06	83,3	84,37	101,3
10000	0,001	10	0,06	166,7	147,63	88,6

Table 4.15. The percentage sulphur after analysis method august.

Concentration (mg/l)	Volume (l) on Leaves	Thiovit (mg) on leaf	Volume (l) acetone	Calculated concentration (mg/l) in sample	Analysed concentration (mg/l)	% Recovery
0	0,001	0	0,06	0	0	-
600	0,001	0,6	0,06	10	9,69	121,2
2100	0,001	2,1	0,06	35	31,40	112,1
4200	0,001	4,2	0,06	70	65,37	116,7
8400	0,001	8,4	0,06	140	121,30	108,3
16800	0,001	16,8	0,06	280	226,81	101,3

Bio-assay on apple scab

The results of the bio-assay of the remaining efficacy of sulphur against apple scab show a decrease with increasing amount of artificial rain as expected (figure 4.6). It is noted that plants treated with both types of sulphur and no rain yet had a low level of apple scab, whereas a full control was expected. This means that the full rate of sulphur could not prevent infestation by apple scab under these conditions.

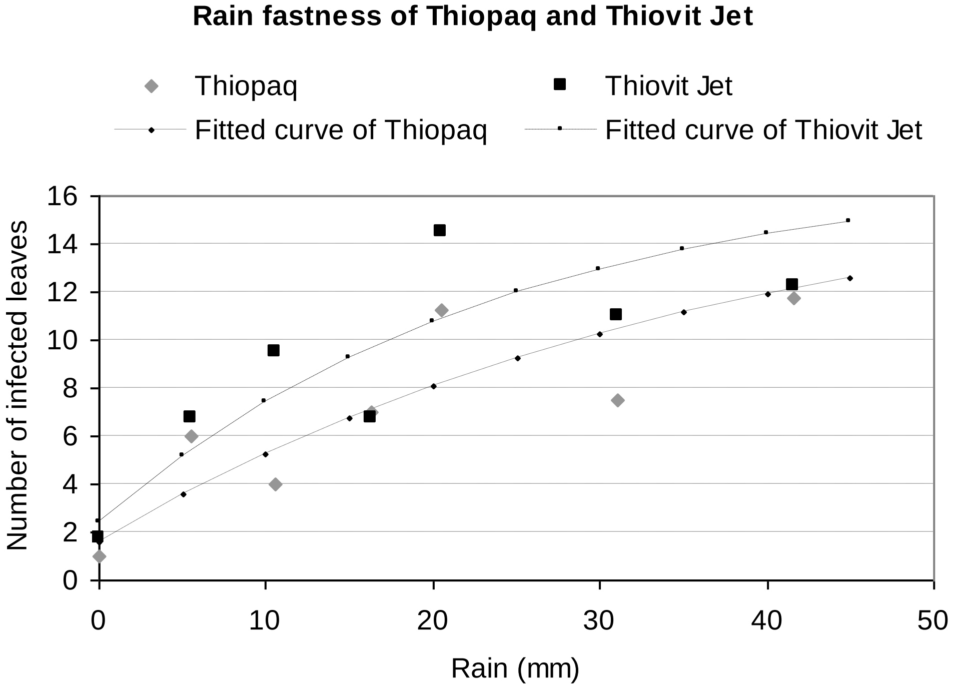

Figure 4.6. The average scab incidence (leaves per plant) of plants treated with either Thiopaq sulphur of Thiovit Jet after different amounts of artificial rain.

The efficacy of Thiopaq sulphur was just not significantly different from that of Thiovit Jet (*P* = 0.054) for scab incidence expressed as the number of leaves infected (figure 4.6). However, looking at scab severity expressed as the number of lesions (spots) of scab per pot than Thiopaq sulphur was significantly more effective than Thiovit Jet (*P* = 0.016; figure 4.7). This is similar to the results on the efficacy against mildew of cucumber. It was not possible to calculate a model for these data due to too high variance. For example, remaining sulphur after 20 mm or rain was less efficacious than that remaining after 30 and 40 mm of rain for both types of sulphur. The no-rained plants had 18.78 leaves infected per pot. This is more than at 40 mm of rain. This means that even after 40 mm of rain, a small amount of sulphur residue is left, for some efficacy. It is noted that infestation doubled already at 5 mm rain.

The infestations were also calculated as the number of leaves infected per shoot in stead of per pot. In this case (figure 4.8), Thiopaq sulphur was significantly more effective than Thiovit Jet. All average data are presented in Table 4.16.

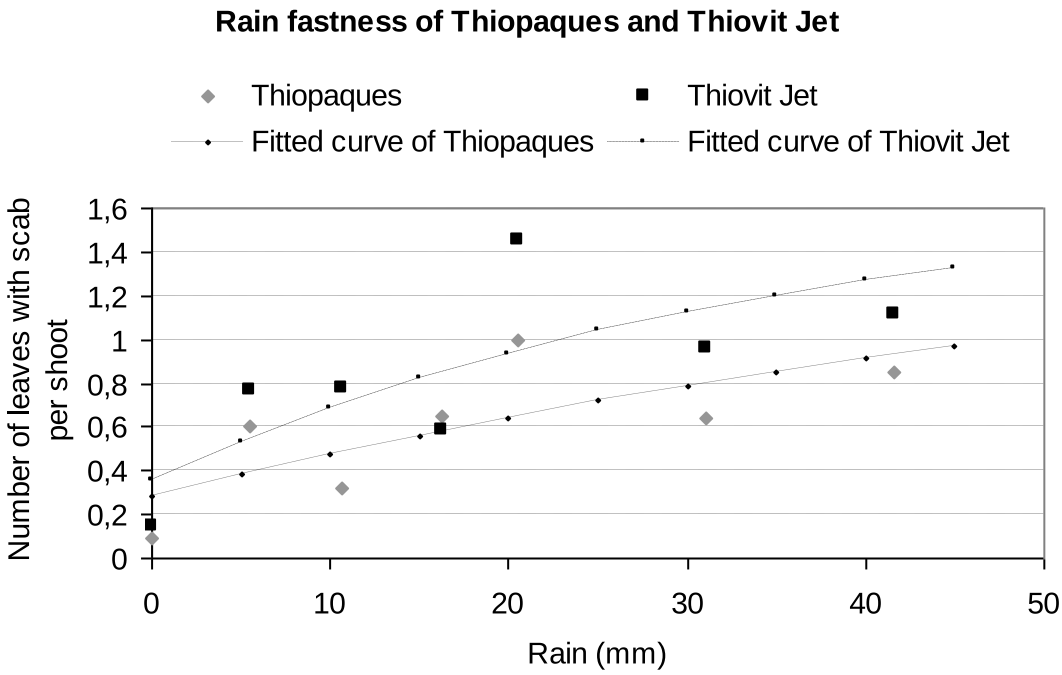

Figure 4.7. The average scab severity (lesions per pot) of plants treated with either Thiopaq sulphur of Thiovit Jet after different amounts of artificial rain.

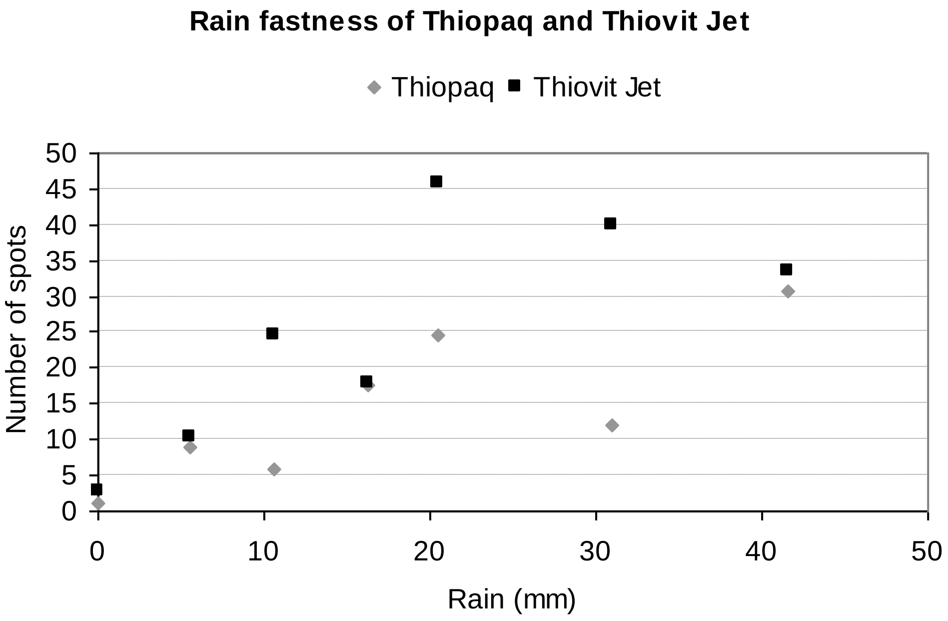

Figure 4.8. The average number of leaves with scab per shoot of plants treated with either Thiopaq sulphur of Thiovit Jet after different amounts of artificial rain.

Table 4.16. Average number of leaves with scab per shoot and per pot and the average number of scab lesions per pot of plants treated with either Thiopaq sulphur or Thiovit Jet after different amounts of artificial rain.

treatment	sulphur type	rain (mm)	no. leaves with scab per pot	no. leaves with scab per shoot	no. lesions per pot
1	Untreated	0.0	20.00	1.89	54.00
2	Thiovit Jet	0.0	1.75	0.13	2.75
3	Thiovit Jet	5.5	6.75	0.77	10.25
4	Thiovit Jet	10.6	9.50	0.77	24.50
5	Thiovit Jet	16.3	6.75	0.62	17.75
6	Thiovit Jet	20.5	14.50	1.40	45.75
7	Thiovit Jet	31.0	11.00	0.92	40.00
8	Thiovit Jet	41.6	12.25	1.16	33.50
9	Untreated	0.0	16.75	1.51	52.00
10	Thiopaq sulphur	0.0	1.00	0.09	1.00
11	Thiopaq sulphur	5.5	6.00	0.60	9.00
12	Thiopaq sulphur	10.6	4.00	0.31	5.75
13	Thiopaq sulphur	16.3	7.00	0.64	17.50
14	Thiopaq sulphur	20.5	11.25	0.99	24.50
15	Thiopaq sulphur	31.0	7.50	0.67	12.00
16	Thiopaq sulphur	41.6	11.75	0.81	30.75

The remaining quantity of sulphur per square m leaf was related to the quantity of artificial rain (figure 4.9 and Table 4.17). The quantity of remaining sulphur per m^2 leaf decreases as the quantity of increases. However, the decrease seems less that the biological efficacy of the remaining residue. The levels of Thiopaq sulphur are significantly lower that those of Thiovit Jet. The rate of decrease is similar for both types of sulphur.

Table 4.17. Average quantity of sulphur per m^2 leaf with increasing amounts of artificial rain.

treatment	sulphur type	rain (mm)	sulphur on leaves (g/m^2)	% with respect to untreated
1	Untreated	0.0	0.00	-
2	Thiovit Jet	0.0	0.81	100.0
3	Thiovit Jet	5.5	0.70	86.8
4	Thiovit Jet	10.6	0.60	74.2
5	Thiovit Jet	16.3	0.63	78.4
6	Thiovit Jet	20.5	0.56	69.5
7	Thiovit Jet	31.0	0.65	80.4
8	Thiovit Jet	41.6	0.60	74.8
9	untreated	0.0	0.00	-
10	Thiopaq sulphur	0.0	0.66	100.0
11	Thiopaq sulphur	5.5	0.49	74.0
12	Thiopaq sulphur	10.6	0.52	79.6
13	Thiopaq sulphur	16.3	0.48	73.1
14	Thiopaq sulphur	20.5	0.47	71.9
15	Thiopaq sulphur	31.0	0.35	53.6
16	Thiopaq sulphur	41.6	0.47	72.4

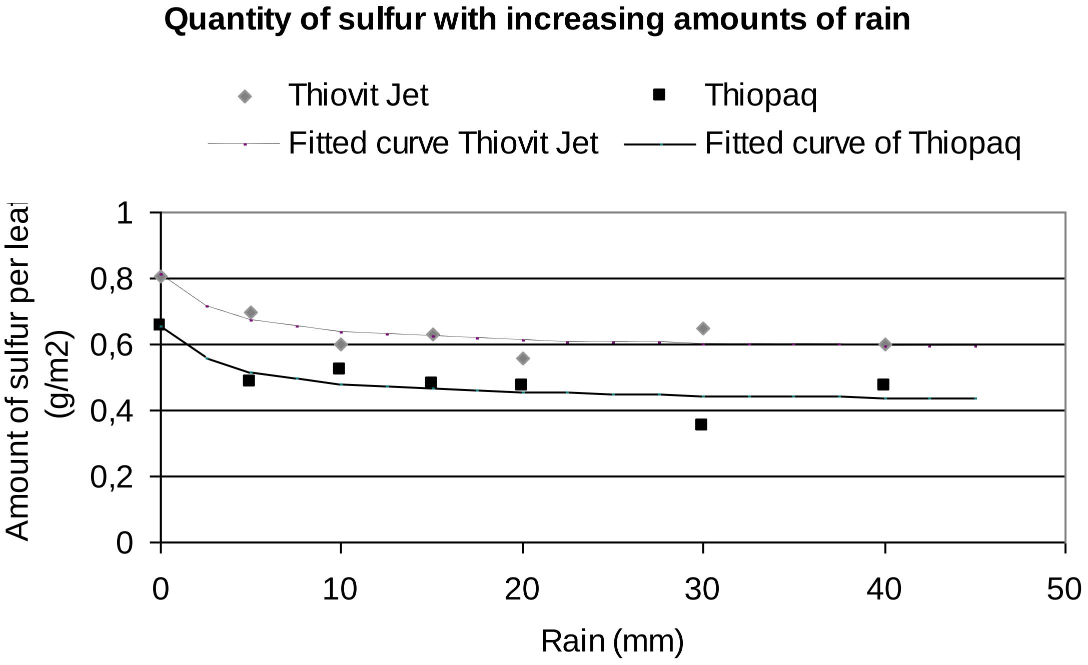

Figure 4.9. The quantity of remaining sulphur per leaf area artificially rained with increasing amounts of tap water.

It is noted that the curves expressing the remaining sulphur for Thiopaq sulphur and Thiovit-Jet are parallel. This suggests a similar rainfastness for both products. It is remarkable that already with a small amount of artificial rain a relative large portion of sulphur washed off the leaves. Subsequently, the quantity of sulphur remaining on the leaves hardly further decreased with increasing quantities of rain.

4.6. Conclusions for Thiopaq™ biosulphur as pesticide

- Thiopaq sulphur was more effective in the control of apple scab and mildew without surfactant added than Thiovit Jet
- Thiovit Jet controlled apple scab for 87 % and reduced spore germination for 93 % at 8 °C in this experiment
- Either type of sulphur provided no complete control of apple scab under these conditions.
- Infection doubled at 5 mm of rain.
- Not all sulphur was washed off by 40 mm of rain.
- Remaining residue of Thiopaq sulphur was lower than that of Thiovit Jet.
- Rain fastness of Thiopaq sulphur and Thiovit Jet was similar.

4.7. Discussion and outlook

The results from the experiments done in this KIEM-EET-project clearly demonstrate that Thiopaq has high potentials as a fungicide. However, the results also reveal some not understood properties of Thiopaq. Some of these, which might influence the market potential as a fungicide, are discussed hereafter.

Apart from sulphur, the product Thiopaq in itself contains substantial amounts of salts, mainly bicarbonates. These salts derive from the process in the reactor, which is kept at high pH-values with hydroxides. For that purpose, either sodium or potassium hydroxide are suitable. Both sodium and potassiumbicarbonate are sometimes applied to crops with the aim to activate the plants defence mechanisms in order to achieve a reduced susceptibility for diseases. This is described in “grey” literature. A careful conclusion from these sources suggests that potassiumbicarbonate has the better biological efficacy against apple scab and sodiumbicarbonate is more biological efficient against powdery mildew diseases. Background information about the role of sodium and potassiumbicarbonate and the influence on the quantity of these salts in Thiopaq are essential for understanding the fungicidal activity of Thiopaq. This knowledge should help the positioning of Thiopaq in the fungicide market.

In this KIEM-EET-project, it has been demonstrated that there are remarkable differences between Thiopaq and the reference fungicide Thiovit Jet in the binding forces to different surfaces. The binding forces to plant tissue is an important factor for the use of sulphur as a fungicide. The binding force to the plant tissue determines the rainfastness of the product. More information is needed to answer the ever returning question of users, after how much rainfall re-application is necessary. Additives or other mechanisms to improve the rainfastness should be searched for.

However, care should be taken with additives. Addition a surfactant decreased the efficacy of Thiopaq against mildew in cucumber. This is not understood and should be clarified.

5. BIOSULPHUR AS FERTILIZER

A sustainable form of agriculture, i.e. one which does not simply exploit the inherited fertility of agricultural soils without any thought for the future, is based on the premise that plant nutrients which are removed with the harvested crops will be replaced. It is the regular supply of sufficient quantities of plant nutrients which has, over the past 100 years, maintained and even improved the fertility of farmers’ fields in Europe. Combined with advances in plant and animal breeding, plant protection, farm mechanisation and land management, plant nutrition has been responsible for an increase in EU farm output in recent years, in spite of a reduction in the total agricultural area.

Plant nutrients are available from four different sources:

- the soil itself,
- livestock, municipal and industrial wastes,
- biological nitrogen fixation, and
- mineral fertilizers.

Mineral fertilizers allow farmers to supplement the nutrients, which are already present in the soil or provided by organic manures and legumes, and thus match the supply of nutrients with the needs of the crops. They are, in fact, the only nutrients, which can be tailored to meet the crops' exact requirements. Providing guaranteed contents and the possibility of application as and when required, mineral fertilizers are a cost-effective means of achieving sustainable crop production and improvements in the quality of food and fodder.

Sulphur is regarded as secondary nutrient although plant requirements for sulphur are equal to and sometimes exceed those for phosphorus a primary nutrient. However sulphur is recognised as one of the major nutrients essential for plant growth, root nodule formation of legumes and plants protection mechanisms. Sulphur deficiency has become widespread in many countries in Europe. Because atmospheric inputs of sulphur will continue to decrease, the deficit in the sulphur input/output is likely to increase, unless sulphur fertilisers are used.

5.1. Plant Nutrient Requirements

Plants require nutrients in order to grow, develop and complete their life cycle. The supply of nutrients to the plants should be balanced in order to maximise the efficiency of the individual nutrients so that these meet the needs of the particular crop and soil type. The primary, or macro, nutrients required by plants are:

Carbon	C	Nitrogen	N
Hydrogen	H	Phosphorus	P
Oxygen	O	Potassium	K

Carbon, hydrogen and oxygen, which, together with nitrogen, form the structural matter in plants, are freely available from air and water. Nitrogen, phosphorus and potassium, on the other hand, may not be present in quantities or forms sufficient to support plant growth. In this case, the absence of these nutrients constitutes a limiting factor.Nitrogen is an essential component of amino acids and, therefore, of proteins which include nucleic acids, enzymes, and the green, light-harvesting pigment, chlorophyll. It is also the nutrient, which normally produces the greatest yield response in crop plants.

Phosphorus is an essential part of the enzymes, which help the crop to fix light energy. It forms an integral part of nucleic acids, the carriers of genetic information, and is important in stimulating root growth. Potassium is involved in processes, which ensure carbon assimilation and the storage of sugars and proteins. The potassium ion is also important for water regulation and uptake. Furthermore, the presence of potassium in sufficient amounts ensures resistance to frost, drought and certain diseases.

In addition to nitrogen, phosphorus and potassium, there are other nutrients, which are essential for plant growth. Commonly known as secondary nutrients, these are:

Sulphur	S	Chlorine	Cl (not always essential)
Calcium	Ca		
Magnesium	Mg	Sodium	Na

Magnesium occurs in chlorophyll and is also an activator of enzymes, while sulphur forms part of two essential amino acids, which are among the many building blocks of protein. It is also found in vitamin B1 and in several important enzymes. Sulphur is also essential for root nodule formation of legumes and is recognised as important for plant protective mechanisms either trough its presence in biocidel plant compounds e.g. glucosinolates and alliins, and through the emission from leaves of volatile sulphur compounds which have fungicidal effects. Calcium is required for plant growth, cell division and enlargement. The growth of root and shoot tips and storage organs is also affected by calcium as it is a component of cell membranes. Calcium is also vital for pollen growth and to prevent leaf fall.

There are also several micro nutrients which are essential for plant growth. These are:

Iron	Fe	Molybdenum	Mo
Zinc	Zn	Boron	B
Manganese	Mn	Cobalt	Co
Copper	Cu		

Although plants receive a natural supply of nitrogen, phosphorus and potassium from organic matter and soil minerals, this is not usually sufficient to satisfy the demands of crop plants. The supply of nutrients must therefore be supplemented with fertilizers, both to meet the requirements of crops during periods of plant growth and to replenish soil reserves after the crop has been harvested.

5.2. Sulphur deficiency

Sulphur has been recognized as an essential element for plants for more than two centuries, but only in the last decades has S deficiency emerged in agricultural crops.

The sulphur reserves in Europe soils have been dropping for the last 30 years. In the past, 33% to 50% of the sulphur in soils came from rainfall, but with less air pollution there is now less sulphur in rainfall. In the 1950s fertilisers routinely contained sulphur but this changed in the 1960s. As a result the amount of sulphur reaching the soil has diminished.

Figure 5.1 shows the expected S deficiency in the world.

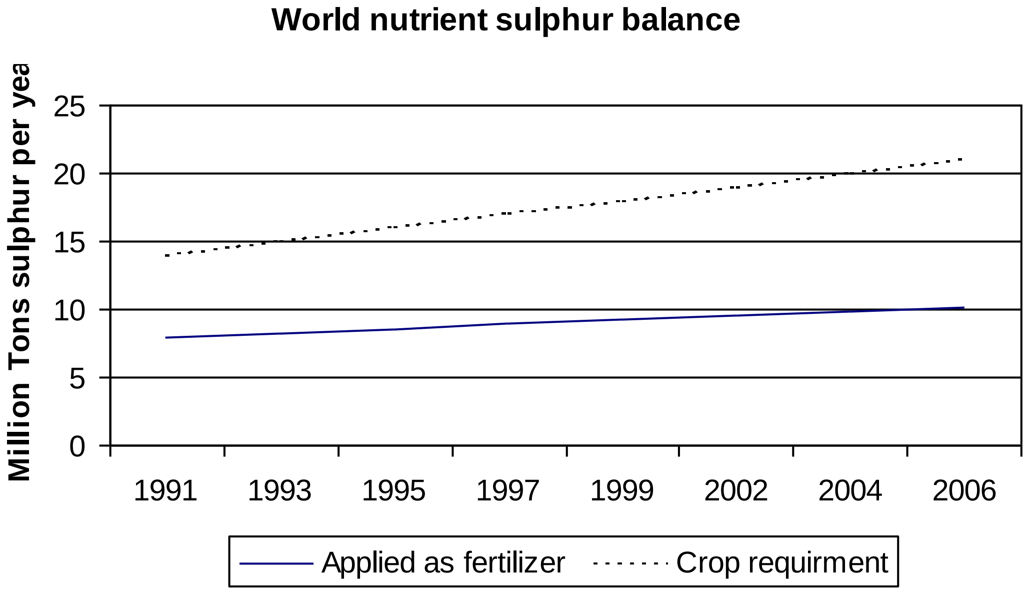

Figure 5.1 World nutrient sulphur balance. 2006-2008 Sulphur deficit > 10 million Tons ref: IFA

The symptoms of sulphur deficiency are cupped shaped and deformed foliage, which is frequently stiff. The symptoms will be more defined where there is poor soil structure or compaction, which restricts root growth, resulting in the plant being unable to take up sufficient sulphur.

Sulphur is required by the plant to make amino acids and to utilise nitrogen. The typical nitrogen deficiency symptoms of a purple flush on leaves and midribs can usually be seen in sulphur deficient plants. Multi-cut grass for silage has been shown to be highly susceptible to sulphur deficiency, and yield responses to sulphur of between 5 and 30% are common. For cultivation of fodder crops (grasses) in the Netherlands an official Sulphur fertilize recommendation is given by “Commisie Bemesting Grasland en Voedergewassen”.

Oilseed rape is also susceptible to sulphur deficiency, and sulphur fertilisation can produce a dramatic yield response under deficiency conditions. Sulphur deficiency in oilseed rape has been widely reported in France, Germany, Denmark and the UK. Although cereals have a lower requirement for sulphur, yield responses to sulphur application of between 5 and 30% have been obtained in France, and also increasingly in the UK and Germany. In general, field trials across different countries have shown that oilseed rape responds to sulphur applications up to 30 kg S/ha, cereals up to 20 kg S/ha, and grass between 20 and 40 kg S/ha.

Sulphur deficiency not only affects yield, but also impacts on crop quality. It has been demonstrated that the sulphur status of wheat grain has an important influence on bread-making quality. Forage grasses low in sulphur are nutritionally inferior for animals. Maintaining a sufficient sulphur status also has a positive effect on the quality of legumes and sugar beet. Occurrence of sulphur deficiency is more likely to be found on light or soils with low organic matter content, in areas of low atmospheric deposition of sulphur and with excessive winter rainfall.

5.3. Sulphur recommendations for Europe

Soil analytical techniques to predict the need for sulphur are not reliable. Analysis of soil S gives a poor prediction of likely responses to S-based fertilizers. The reason for this is that most S retained in the soil occurs in the organic matter which must be broken down for S to be released. The mineralisation is effected by soil temperature and moisture availability which are difficult to predict, in turn making prediction of quantity and timing of S released difficult to predict. Plant sulphur content can be a useful guide to the adequacy of sulphur nutrition. This in combination with Plant N:S ratios can be a helpful indicator of likely responds to applied S. Although tissue analysis provides a more accurate guide of the sulphur nutritional status of a crop plant, the optimum time for accurate assessment is often later than the optimum time for S supplementation. This may inhibit the usefulness of this more accurate assessment system. Consequently most advisory bodies across Europe adopt an empirical system of advising the level of S required based on crop, potential yield, soil type and farming system.

An example of S recommendation for grassland on sandy soils in the Netherlands is presented in table 5.1.

Table 5.1 Netherlands; *Sulphur fertiliser recommendations for grassland on sandy soil* Source:Adviesbasis bemesting grasland en voedergewassen November 2002 Commissie Bemesting Grasland en Voedergewassen

SLV* Kg S/ha	Classification	1st Cut Kg S/ha	2nd cut Kg S/ha
< 6	Very low	20	20
6 –11	Low	15	15
12 –17	Fairly Low	0 -15	15
> 17	Sufficient	0	0
>23	High	0	0

* The Sulphur Supplying Capacity (SSC or SLV) during the first three cuts (until mid July) is calculated from the total S content on the 0-10 soil layer: SSC (kg S/ha) = 17,8 x S-total (g/kg) x density of the soil

The International fertiliser society (ifs) can provide additional information on S recommendations of 17 European countries. In general, levels of S recommended vary considerably with country and with the type of organisation providing the advice.

5.4. Sulphur fertilizer types and method of application

Sulphur fertilisers are most commonly available in either the soluble sulphate or elemental forms. The products containing ammonium sulphate predominate the marketplace whereas products comprised of elemental Sulphur, are only recently finding increased market acceptance.

Provided there is adequate soil moisture, ammonium sulphate fertilizers quickly dissolve to release SO4-S (the sulphate form). SO4-S is highly mobile in the soil and is the only form of Sulphur directly utilized by plants. Because ammonium sulphate fertilizers are so rapidly available, they can be applied to crops in many different ways.

If detected early enough, Sulphur deficiency can be corrected in many crops by broadcasting ammonium sulphate. The greatest disadvantage and risk from using sulphate fertilizers are leaching, i.e. sandy soils subjected to heavy rainfall. Under these conditions, SO4-S may leach below the rooting zone.

For fertilizer producers, elemental sulphur (S) has the advantages of ready supply, lower production and transportation cost and are less susceptible for leaching. Despite these benefits, the use of S fertilizers is limited because it takes considerably more time for S to become available compared to soluble sulphate forms of fertilizer. The rate of conversion from S to plant available SO4-S mainly depends on the particle size to which the product degrades and the method of application.

The evolution of S products is rapid. Some of the products available are Tiger 90cr, Sulpher 95, Montana 90, Keg River 85, Brimstone 90 and Luxan Sulphur 90). The industry is continually striving to improve the dispersing nature of their S products. Research has determined that S granules that break down into particles smaller than 150 Microns convert quickly, since particles of this size (and smaller) provide sufficient surface area for microbes to act. Regardless of brand, all commercial S products should be surface broadcast without immediate (or no) incorporation for at least a year before the crop. Elemental sulphur present on the soil surface is more easily oxidized by soil microbes because rainfall and freeze/thaw cycles break down and disperse the granules. Banding or immediate incorporation is an inefficient method of applying S. The surrounding soil keeps the S granule intact, reducing the exposed S surface area and vastly reducing the conversion rate to SO4-S. Even with broadcast applications, the rate of conversion from S to SO4-S can vary from less than a year to several years or more. Instead of relying on applications of elemental S alone, a good option would be to supplement with applications of ammonium sulphate until the elemental S has had time to break down.

5.4.1. Animal manure as S fertilizer

Animal manure is low in S relative to nitrogen. It has been observed that N:S ratios varied from 7:1 to 17:1 for cattle manure and from 13:1 to 25:1 for pig manure. The majority of these N:S ratios are too great for optimum crop production. In the majority of cases, supplemental S fertilization will be required when using animal manure to fertilize.

5.4.2. Bio-sulphur as fertilizer

Bio sulphur produced from Thiopaq (biogas) gas purification plants is highly suitable as S fertilizer due to it high purity and absence of pollutions. Bio sulphur can also be produced from Thiopaq processes treating wastewater or process water streams, converting sulphate or sulphuric acid in to elemental bio-sulphur.

The hydrophilic properties of the bio-sulphur and its small particle size (less than 20 µM after drying) should offer agronomic advantages for short season climates affected by sulphur deficiency. In greenhouse and field experiments the applicability of Thiopaq bio-sulphur as a soil fertilizer was evaluated by the Alberta Agricultural Research Institute, University of Edmonton. For this purpose, the effect of biologically produced sulphur on the

Canola yield was compared with the effect of several commercially available sulphur fertilizers. The results look very good for the biologically produced sulphur. Compared with reference test (without any sulphur added) the growth simulation, due to bio-sulphur, is very significant with a yield of 50 % being observed. This result places the bio-sulphur among the best of presently produced soluble sulphate and S fertilizers.

5.5. Market potential of sulphur fertilizers

About 9 million tons of sulphur, equal to about 15 % of total world sulphur consumption, are applied annually in the form of ammonium sulphate and compound fertilizers. The marketplace is currently dominated by ammonium sulphate fertilizers, which cost less per tonne. However, S fertilizer products are gaining a larger market share. Since they contain larger percentages of Sulphur, S fertilizers actually cost less per kilogram of Sulphur.

As presented in figure 5.1 the current worldwide deficit for sulphur is approx. 9,5 million tons. Fertilizer producers in America, Europe and Asia are producing direct application materials based on elemental sulphur to satisfy the sulphur deficit and seize market opportunities. Assuming that 15 % of projected deficit is captured – this being a conservative estimate – S fertilizer demand could increase by 1,5 million tons per year over the next decade.

Published farm gate prices for S fertilizers (e.g. Brimstone 90) are varying between € 200,- and € 250,- per ton S, resulting in an additional revenue for the fertilizer industry of more than € 300 million annually. This vast market potential for S fertilizers could benefit the applicability of Paques Thiopaq technology. Based on recent studies the produced Thiopaq bio-sulphur shows to be an excellent sulphur fertilizer. Due to the small particle sizes an optimal conversion rate to sulphate, already during the first year of broadcasting, seems possible.

Until now the amount of produced bio sulphur is relatively small. The total annual production of Thiopaq bio-Sulphur is approx 7.000 tons, which is < 0.1 % of the present sulphur deficiency in the world on an annual base.

For the Netherlands, based on the S recommendations for grassland, the total expected demand of S fertilizer will be ranging between 10.000 - 35.000 ton/year. The total annual production of bio-sulphur in the Nederland is 185 tons, which represent 2 % of this S demand.

5.6. Conclusions

The current amount of produced bio-sulphur is not enough to play an important role as S fertilizer. For the moment the combination of locale production and locale S-fertilizer demand is probably the only feasible way of using bio-sulphur as fertilizer. To gain a market share, significant amounts of bio- sulphur will have to be produced.

The main application for producing high quantities of Bio-sulphur is in the petrochemical industry, especially in the natural gas, flue gas and refinery gas treatment. Legislation and government approval for using bio-Sulphur as fertilizer is also one of the main issues to be solved. Additional field tests and research are necessary to convince governments and agriculture advising bodies on the advantages of using bio-sulphur as S-fertilizer.

Partnership(s) with fertilizer producer(s) could be essential to get these permissions and to create an outlet/market for bio-sulphur.

A significant market for bio-sulphur as fertilizer is likely to be created after investigation of possible partnerships with fertilizer producers on one side and potential Paques clients for Thiopaq technology on the other. The field test result in Canada looks very promising and therefore we believe a market/outlet of bio-sulphur to meet locale S demands for agriculture purposes is possible.

The main role of Paques and his Partners (e.g. Shell) is to act as a technology supplier (Thiopaq) and intermediary.

5.7. Literature

- Shell/Paques design manual: Biological H_2S oxidation-version 1.0
- Kerr Walker and Chris Dawson 2002. Sulphur fertilizers recommendations in Europe Proceedings No. 506. International Fertilizer Society. York UK

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6. ECONOMICAL PERSPECTIVES

In this chapter a cost prize calculation of Thiopaq™ Biosulphur is given for different scenarios. Thereafter marketing aspects of biosulphur as fungicide or fertilizer are discussed.

6.1. Cost prize calculation for THIOPAQ biosulphur

Thiopaq™ biosulphur competes with Claus sulphur and other sulphur sources. Although the biosulphur has some unique characteristics like the particle size and hydrophilic nature the cost prize of the sulphur should be as close as possible to the cost price of competing sources. Cost prizes are calculated for the decanted sulphur slurry (60 wt% solids of which 97% sulphur) and the dried product (92 wt% solids of which 97% solids).

6.1.1. Types of sulphur producing aplications

Thiopaq™ biosulphur is produced by two types of applications:

1. biogas scrubbers.
2. natural or refinery gas scrubbers.

Applications of the first type are generally small in scale. Sulphur production is between 50 and 300 kg sulphur per day. The sulphur is produced as a 15wt% slurry. This slurry is normally sent to the aerobic waste water treatment facility where it is oxidized to sulphate. Some applications already have a decanter centrifuge to concentrate the sulphur to a 60 wt% solids slurry. The slurry is collected in 200 kg containers and normally sent to a waste dump. The biogas applications are mainly concentrated in the Netherlands and Germany.

Applications of the second type are normally of a larger scale. Sulphur production is between 1 and 15 tons of sulphur per day. The sulphur is produced as a 60 wt% solids slurry or as a 92 wt% solids powder. The slurry is normally collected in open top containers (3 – 4 m^3) and sent to a waste dump. The powder is collected in 1 ton big bags and sold as fertilizer. These applications can be found where oil and gas is produced. Two plants are located in Canada, one in Egypt, two are built in the USA and one in Australia.

6.1.2. Cost price calculation for biogas applications

The only way to get sufficient sulphur together is to collect the sulphur produced at a number of customers. The sulphur can then be processed together. At the moment about 1 ton of sulphur is produced each day at 6 locations that are within 300 km of Balk (location of Paques head quarters). This sulphur is used in the cost price calculations. The amount of sulphur is expected to increase to 3 tons of sulphur per day in 2005. Because the new plants that are coming on stream have the same size at similar locations, the cost price calculated here is representative for the expected future cost price. The cost price is compared to the cost of the current disposal method. Preferential method of disposal is a dump site. However, as legislation in Germany is changing in 2005, dumping sulphur will no longer be allowed. Therefore the cost of alternative disposal methods like deep mine disposal and incineration is also given.

Table 6.1: Six customers used for cost price calculation.

Customer:	Amount of sulphur produced per day	Form:
Zulpich Paper	200 kg	60 wt% solids slurry
Eerbeek	130 kg	15 wt% solids slurry
De Wierden	30 kg	15 wt% solids slurry
Palm Wörth	250 kg	15 wt% solids slurry
Stone Europe	200 kg	15 wt% solids slurry
Lamb Weston	50 kg	15 wt% solids slurry

Cost of decanter centrifuge step

Three scenarios were developed:

1. Sulphur is collected in current output form in totes (normally referred to as multiboxes) and transported to Balk.
2. Decanter centrifuges are installed at all customers. Sulphur is collected in multiboxes in decanted form and transported to Balk.
3. Sulphur is collected and stored at customer site. A mobile containerized decanter centrifuge unit concentrates sulphur during site visit. Sulphur is collected in multiboxes and sent to Balk.

Table 6.2: Cost price calculation for scenario 1 per ton of sulphur (EURO).

Customer	Transport cost	Decanter cost	Total cost	Current disposal cost	Expected disposal cost
Zulpich Paper	119	-	119	80 - 100	600
Eerbeek	78	-	78	150	150
De Wierden	29	-	29	167	167
Palm Wörth	228	-	228	80 - 100	600
Stone Europe	194	-	194	80 - 100	600
Lamb Weston	156	-	156	167	167

Table 6.3: Cost price calculation for scenario 2 per ton of sulphur (EURO).

Customer	Transport cost	Decanter cost	Total cost	Current disposal cost	Expected disposal cost
Zulpich Paper	119	-	119	80 - 100	600
Eerbeek	40	256	297	150	150
De Wierden	15	1111	1126	167	167
Palm Wörth	114	133	247	80 - 100	600
Stone Europe	97	167	264	80 - 100	600
Lamb Weston	78	667	744	167	167

Table 6.4: Cost price calculation for scenario 3 per ton of sulphur (EURO).

Customer	Transport cost	Decanter cost	Total cost	Current disposal cost	Expected disposal cost
Zulpich Paper	119	-	119	80 - 100	600
Eerbeek	40	101	141	150	150
De Wierden	15	101	116	167	167
Palm Wörth	114	101	215	80 - 100	600
Stone Europe	97	101	198	80 - 100	600
Lamb Weston	78	101	179	167	167

Cost of drying step

The slurry can be upgraded further to a powder. Thanks to the kind cooperation of Hosokawa Micron B.V. the costs of the drying step could be calculated.

The calculations are based on the use of a Hosokawa paddle dryer that is also used at the THIOPAQ installation in Egypt. The dryer can be installed in a 20 ft sea container. It can be operated on a daily shift basis (7 hrs per day, capacity 1 ton per day) and / or continuous basis (capacity 3 tons per day). An estimate was also made for the investment and operational cost of the 13 ton powder a day drier that is operating at the THIOPAQ installation in Egypt. The cost price for the powder produced with this dryer is compared to the estimates prepared by Hosokawa.

The cost price calculation includes the initial capital investment (write off period equal to 7 years), all utilities and manpower based on 1800 operating hours per annum.

Table 6.5: Cost price for drying step

Dryer capacity Ton S/day	Total cost per annum €/annum	Unit cost € / ton S
1	147 900	670
3	314 750	430
14	2 999 743	27

Total sulphur upgrading cost for biogas applications

From the three scenarios for the decanter centrifuge step, the mobile containerized option is most economically viable with cost per ton of sulphur in the 80 to 167 € / ton S range. Drying cost for the capacity add up to another 670 € / ton S.

Depending on the required form of the sulphur product, the preferred form from a cost price point of view would definitely be the slurry form with a cost price of 80 to 167 € / ton S. Cost price for the powder would be 750 to 837 € / ton S.

The effect of scale can already be seen in the cost of the drying step. As soon as enough biologically produced sulphur can be collected, costs decrease considerably. For example for the 3 ton S per day produced case, cost price for the powder drops to between 510 and 597 € / ton.

6.1.3. Cost price calculation for natural gas applications

The determination of the cost price for natural gas application is much simpler as they already have the decanter centrifuge installed. The only option then is consider further upgrading to a powder. As the quantities of sulphur produced are much larger, the economics should also be getting more attractive. Capacities of the natural gas application range between 1 and 14 tons per day.

Cost of decanter centrifuge step

The decanter centrifuge is already part of the initial investment decision. No additional investment cost have to be made to get the decanted slurry. The cost of the decanter centrifuge therefore does not have to be taken into account in the sulphur disposal cost.

Cost of drying step

The cost of the drying step is the same as is given in table 6.5. The only difference would be that the owners of the plant would have a choice between one or three shift operation. Since it is expected that full time operation on an 8000 operating hours per annum basis would be preferred, the cost for the 3 and 14 ton S per day driers are more representative than the 1 ton.

It is also expected that the natural gas plant operators would not provide full time manpower. The drier would be engineered in such a way that it would operate fully automated. Initial investment cost would increase slightly but operational cost would drop. Taking these assumptions into account, the adjusted powder cost prices are shown in table 6.6.

Table 6.6. Cost price for drying step

Dryer capacity Ton S/day	Total cost per annum € / annum	Unit cost € / ton S
1	113 000	513
3	210 000	290
14	2 999 000	27

Total sulphur upgrading cost for natural applications

The cost of sulphur upgrading for natural gas plants is more attractive as they have a decanter centrifuge installed in the standard line up. Due to a higher degree of automation, operating cost of the dryer step are also expected to change.

Since the influence of scale is also significant, the collection of the sulphur of different plants might also be considered for these larger scale plants.

6.2. Marketing of THIOPAQ biosulphur as fungicide and fertilizer

6.2.1. The Dutch fungicide market

Based on the testing result gained in this EET-study, different companies were contacted. These companies are active in the market of fungicides :

1. EcoStyle (and its German partner Neudorff)
2. AAKO
3. Syngenta
4. BASF

Based on the discussions with representatives of these companies, the following can be said about the possibilities of THIOPAQ biosulphur as fungicide:

The Thiopaq™ sulphur is biologically produced and is therefore very suitable for the application in the sustainable agriculture market. This market is showing signs of consistent growth and commercial attractiveness. The companies see the biosulphur as a sexy alternative with strong possibilities; not only from a marketing perspective but also because the results of the first tests look very promising. Because the Thiopaq™ biosulphur is a waste product, cost price is expected to be low.

Before Thiopaq™ biosulphur can be applied as fungicide, it needs to be approved by the Dutch Government for use as a fungicide. The approval process requires the build up of a dossier which contains the results of extensive testing. Because the Dutch government is responsible for the approval process, it needs to make sure that the Thiopaq™ sulphur would be an excellent fungicide and is not harmful to the crops, the environment and the consumers. A number of tests was defined to be able to develop enough knowledge about the new product. The testing process is both extensive, expensive (1 – 2 million Euro) and lengthy (2 – 4 years).

The length of the approval process creates a lot of uncertainty.

- Will the market still exist in a number of years ?
- Will the product pass all the tests ?

A company should be make sure that it has done its homework before it starts the approval process. More uncertainty is added because a change in legislation is expected. At the moment the product would need to be approved in each country separately. A dossier that was approved in for instance the Netherlands can be used in other countries with some additional effort required. European legislation is expected within the next couple of years. This development creates another cause of uncertainty for a company that would like to start with the development of a dossier.

An alternative would be to market the new product under the wings of a company that already has sulphur approved as a fungicide component. Discussions will need to be opened with the companies that could develop the Thiopaq™ biosulphur as a new alternative under the protection of their current dossier. Next to the companies in the list above, the following companies can also be approached:

1. Luxan
2. Adrichem

Way forward

The search for a partner needs to be concluded and the Thiopaq™ biosulphur needs to be developed further into an attractive active component of a commercial fungicide. Because the approval process for fungicide application is lengthy, costly and uncertain from a legislation point of view, it should be preferred to cooperate with a partner that has already obtained approval.

6.2.2. The Dutch fertilizer market

The potential of THIOPAQ biosulphur for fertilizer applications was discussed with:

1. Ecostyle.
2. Flex Fertilizer

Previously discussions were shared with Hydro Agri and Fertiva.

Based on Dutch legislation and the two year test in Canada on canola yield with biosulphur as fertilizer, it should be relatively easy to get approval for fertilizer application. After approval, a series of field tests can be organized for marketing purposes. The next season the sulphur could be sold commercially. A field test generally costs between € 5000 and € 10000 and can be organized by any recognized institute.

Both companies named the low prices for sulphur based fertilizer as the biggest hurdle for success. Because the product is biologically produced and has an extremely small particle size, a good potential is seen in certain niche markets like biological farming.

Way forward
The fertilizer market can be approached relatively easy by field testing. Because fertilizer prices are relatively low, niche markets with slightly higher margins should give the best potential.

6.2.3. Other contacts

At the Sulphur 2003 conference in Canada contact was started with JS Technical Services. This company is an international sulphur trader. Based on the characteristics of the sulphur powder, they saw a number of opportunities:

1. Additive for grass digestion in England.
2. Fertilizer additive in a powder form in a number of markets.
3. Fertilizer additive in a palletized form in a number of markets.

Depending on quantity, quality and cost prize, JS Technical Services would be prepared to trade the sulphur product.

7. DISCUSSIONS AND CONCLUSIONS

Paques BV has developed a biological technology for H2S removal from natural gas and biogas. This technology can also be applied to desulphurize any kind of sulphide polluted gas. The sulphide is removed from the gas with a slight alkaline washing liquid and in a bioreactor this sulphide is converted to elemental sulphur; Thiopaq™ biosulphur. The particle size of the biosulphur is small, less than 50 µm. This small particle size is beneficial in agricultural applications due to the high area with respect to total weight. In this study the application of Thiopaq™ biosulphur as fertilizer and as fungicide has been studied. In order to apply the biosulphur powder, the biosulphur particles suspended in the bioreactor has to be concentrated and the resulting paste has to be dried. Different types of dryers were used and evaluated. The powders themselves were physical-chemical characterized. A market study for both biosulphur as fungicide or as fertilizer has been performed.

The suspended biosulphur from the bioreactor preconcentrated with a gravity settler could easily be concentrated with a conventional centrifuge. A pasta with a weight percentage up to 60% was obtained. Three different types of dryers were selected based on the pasta properties and product requirements: a paddle dryer, a spray dryer and an inert medium spouted bed dryer. Based on the pilot plant test runs performed with the different dryers it is concluded that acceptable operation was obtained with a paddle dryer and an inert medium spouted bed dryer. As the spouted bed dryer produces a powder with a smaller particle size than the paddle dryer, the spouted bed dryer is the preferred choice.

The colloidal and surface chemical properties of dried biosulphur particles have been established and the interaction of biosulphur particles with model and leaf surfaces have been determined. The powder obtained from the pilot plant dryer differed from the native dried biosulphur in 1) specific surface area, 2) contact angles, and 3) methylene blue adsorption. It seems that the polymeric layer at the sulphur surface behaves different. Also the wetting behavior is surprisingly different. On the other hand, the surface charge as obtained from microelectroforesis is similar for the dried and native samples. The biosulphur has lost part of its hydrophilicity, but the biosulphur is still more hydrophilic than claus sulphur.

The particles size distribution is somewhat dependent on the type of dryer. The dispersion flash dryer gives a single size fraction, whereas with the other dryers fractions with a diameter ranging from 16 to 200 µm are observed. In all cases aggregates were detected. By sonification these aggregates can be destroyed and primary particles with a diameter of 2 µm are obtained.

With atomic force microscopy images of biosulphur particles have been obtained. These images show the crystalline character of the biosulphur particles and enable an alternative for determining the diameter of the particles. The diameter of the particles corresponds satisfactorily with the determination of the particles size distribution.

Interaction forces have been measured between sulphur particles and different surfaces both in air and aqueous environment. Most important is, that we can directly monitor the effect of additives on the contact forces. It was shown that the formulated Thiovit particle in water gives an attractive contact force, whereas for the unformulated Thiopaq particles a small repulsion was observed. Thus it can be expected that Thiovit has a better rain-fastness than the unformulated Thiopaq.

The efficacy of Thiopaq™ biosulphur as a fungicide was tested in three types of experiments: 1) germination test of conidiospores of *Venturia inaequalis*, 2) an efficacy test of biosulphur against mildew on cucumber and 3) a rain fastness test with apple scab. It was concluded from these experiments that Thiopaq™ biosulphur was more effective than

Thiovit Jet in the control of apple scab and mildew without a surfactant added. At 8 °C Thiovit Jet controlled apple scab for 87 % and reduced spore germination for 93 %, but either type of sulphur provided no complete control of apple scab under these conditions. From the rain fastness test it is concluded that already at 5 mm of rain the infection was doubled but that not all sulphur was washed off by 40 mm of rain. Rain fastness of Thiopaq sulphur and Thiovit Jet was similar.

The efficacy of Thiopaq sulphur was just not significantly different from that of Thiovit Jet for scab incidence expressed as the number of leaves infected. However, looking at scab severity expressed as the number of lesions (spots) of scab per pot than Thiopaq sulphur was significantly more effective than Thiovit Jet. This is similar to the results on the efficacy against mildew or cucumber.

A market study was performed for biosulphur as fertilizer and as fungicide. For biosulphur as fertilizer it is concluded that there is large market potential as there is an increasing interest for S fertilizers. Field tests, performed in another study, showed the potential of Thiopaq™ biosulphur; an increase in crop yield of 10% compared with commercial fertilizers was demonstrated. However, the current amount of produced bio-sulphur is not enough to play an important role as S fertilizer. For the moment the combination of locale production and locale S-fertilizer demand is probably the only feasible way of using bio-sulphur as fertilizer. To gain a market share, significant amounts of bio- sulphur will have to be produced.

For biosulphur as fungicide the world annual market as a crop protection product is between 180 and 200 million Euro. The total amount used is estimated to be ca. 200,000 tonnes. Sulphur is used in many crops in different amounts around the world. The amounts used vary between 1 kg/ha and 30 kg/ha. The total consumption of sulphur within fruit applications is about 170,000 ton per year. The use in vegetables is lower (22,000 ton per year). Grape uses most sulphur per hectare (almost 16 kg per hectare) and is the largest consumer of sulphur in the world (120,000 ton per year). The most important use for this crop is protection against powdery mildew. The demand for sulphur as crop protecting agent will grow based on the trends that 1) there is a growing acreage and production in the developing countries, 2) there is an tendency to a more severe regulation in the EU towards crop protecting agents and 3) there is an growing acreage of fruit in general and grape in particular.

A cost prize calculation was performed for two different types of applications: sulphur produced from biogas and sulphur produced from natural gas. The difference between both applications is the size of the production plant. For the biogas application the most economical viable option is collecting the biosulphur on site concentrating, the biosulphur sludge with a mobile centrifuge, and sending the paste to Balk. Cost will be between € 80 to 167 /ton S. When the paste is dried in Balk, this adds € 670 ton/S for 1 ton/day and € 430 ton/S for 3 ton/day. The preferred form from a cost prize viewpoint is concentrated slurry. For the natural gas application production volumes are larger. A centrifuge is part of the initial investment. The additional costs are only the drier investment (€290 /ton S for 3 ton S/day, and € 27/ ton S for 14 ton S/day).

The results of this study show a great potential for Thiopaq™ biosulphur in agricultural applications. Application as fertilizer and fungicide look promising. The route for further development of biosulphur as fertilizer is different from the route for the development of biosulphur as a fungicide product.

OUTLOOK

Fertilizer

Based on Dutch legislation and the two-year test in Canada on canola yield with biosulphur as fertilizer, it should be relatively easy to get approval for fertilizer application. After approval, a series of field tests can be organized for marketing purposes. The next season the sulphur could be sold commercially. A field test generally costs between € 5000 and € 10000 and can be organized by any recognized institute. Ecostyle and Flex-fertilizer were approached. Both companies named the low prices for sulphur based fertilizer as the biggest hurdle for success. As the product is biologically produced and has an extremely small particle size, a good potential is seen in certain niche markets like biological farming. A significant market for bio-sulphur as fertilizer is likely to be created after investigation of possible partnerships with fertilizer producers on one side and potential Paques clients for Thiopaq technology on the other. The main role of Paques and his Partners (e.g. Shell) is to act as a technology supplier (Thiopaq) and intermediary.

Fungicide

Discussions with representatives of different companies were held. These companies regard Thiopaq™ biosulphur very suitable for the application in the sustainable agriculture market. Moreover, they see biosulphur as a sexy alternative with strong possibilities. However, before Thiopaq™ biosulphur can be applied as fungicide, it needs to be approved by the Dutch Government for use as a fungicide. Such an approval is both extensive and expensive (1 – 2 million Euro) and lengthy (2 – 4 years). This lengthy process creates uncertainties like will the market still exist, and will the product be approved. An alternative would be to market a new product under the wings of a company that already has sulphur approved as a fungicide component.

The search for a partner needs to be concluded and the Thiopaq™ biosulphur needs to be developed further into an attractive active component of a commercial fungicide. Because the approval process for fungicide application is lengthy, costly and uncertain from a legislation point of view, it should be preferred to cooperate with a partner that has already obtained approval.

This project demonstrated clearly the efficacy and high potential of Thiopaq™ biosulphur. In order to transform the biosulphur into a commercial fungicide, research should be employed on fundamental surface and colloid science. Besides that controlled field-testing should be performed. The following aspects deserve attention:

- further characterization of the hydrophilic /hydrophobic nature of the biosulphur particles is a prerequisite for application as fungicide

- use of additives in a product formulation to enhance the adsorption of sulphur to a plant leave. Force measurements between a colloidal probe and a surface by an atomic force microscope have been proven to be a good method for studying the interaction between a particle and a leaf surface. Application of this method to practical systems and leaf surfaces have not only great value for developing a new biosulphur fungicide but is also important from a fundamental physico-chemical point of view. Additives will influence the efficacy of the fungicide in two ways: the direct action of the fungicide, but also the rainfastness of the product. Rainfastness is in important issue as users have to be informed after how much rainfall re-application is necessary.

- biosulphur powder will contain substantial amounts of salts, mainly carbonates. The cation can be either potassium or sodium. Both sodium and potassiumbicarbonate are sometimes applied to crops with the aim to activate the plants defence mechanisms in order to achieve a reduced susceptibility for diseases. This is described in “grey” literature. A careful conclusion from these sources suggests that potassiumbicarbonate has the better biological efficacy against apple scab and sodiumbicarbonate is more biological efficient against powdery mildew diseases. Background information about the role of sodium and potassiumbicarbonate and the influence on the quantity of these salts in Thiopaq are essential for understanding the fungicidal activity of Thiopaq.

- based on discussions with fertilizer traders, it appeared that a pellet is preferred over a powder. Formulation of the powder into a pellet or granule makes it easier to handle and to apply in the field. Surface and colloid chemistry knowledge should be employed to optimise the granulation and pelletization process as well as the redispersion process.

8. PUBLICATIONS

Presentations

16 Januari 2003 Heijne, B., Jong, P.F. Rainfastness of sulphur against apple scab; preliminary results. 10th Apple Scab Workshop, 15-18 January 2003, Sicily, Italy.
17 Januari 2004 Heijne, B., Jong, P.F. van de. Rainfastness of sulphur. 11th Meeting on Apple Scab, Gleisdorf, Austria, 16-18 Januari 2004.
26 October 2004, Zessen, E. van, *et al.*, Application of Thiopaq™ biosulphur in agriculture, Sulphur 2004, Barcelona, Spain

Papers

Jong, P.F. de, Heijne, B., Roelofsen, W. 2004 Zwavel regenvast maar met verlies van effectiviteit. (Sulphur rainfastness, but a loose of efficacy.) *Fruitteelt* **94**(24), 10-11.

Zessen, E. van, *et al.*, 2004. Application of Thiopaq™ biosulphur in agriculture, Conference preprints of Sulphur 2004.

In the January-February issue of Sulphur magazine there will be combined paper reporting on the use of Thiopaq sulphur and Lo-Cat sulphur for applications as a fertilizer and fungicide. The paper is based on the paper presented in Sulphur 2004 conference preprints.

Poster presentation

Keizer, A. de, Goloub, T., P., Characterization of biosulphur fungicide particles and AFM force measurements with model surfaces and plant leaves, 18th Conference of the European Colloid and Interface Society, 19-24 september 2004, Almeria, Spain