# Extremely Acidophilic Sulfur-oxidising Bacteria for Wastegas Treatment

## Introduction

The ability of micro-organisms to be active in extreme environments offers challenges to understanding the fundamentals of microbial function and opportunities for sustainable biotechnology. One group of micro-organisms that can grow and survive under extreme acidic conditions are called acidophiles. Acidophiles have a pH optimum less than pH = 3 of which some of obligate acidophiles, unable to grow at all at neutral pH.

The search for unknown micro-organisms can also open up interesting possibilities for new biological wastegas treatment applications. In biological wastegas treatment systems treating reduced sulfur compounds like hydrogen sulfide and carbon disulfide, sulfuric acid is the final product at complete conversion. This acidic water leaves the biological wastegas treatment system when it is operated without the additional of pH neutralizing chemical such as caustic. When possible, this operation without the addition of chemicals is preferred as caustic can be relatively expensive and adds to operational concerns as it requires safety measures for proper feeding and storage.

The low pH process water can, on the other hand, cause several problems. The acidity increases the stress on many microorganisms resulting in reduced activity as well as incomplete conversion which leads to elemental sulfur accumulation. The large amounts of water required to remove the produced acid from a reactor treating highly concentrated waste gasses can also be a limiting factor for many applications. With extremely acidophilic micro-organisms, many of these problems can be solved.

In our quest to obtain new microorganisms, we enriched different bacterial strains converting reduced sulfur compounds under extreme acidic conditions. We found micro-organisms capable of degrading different reduced sulfur compounds at extremely acidic conditions (pH < 0).

## Microbial survival mechanisms at low pH

Acidophiles use a variety of mechanisms that enables low pH tolerance. Baker-Austin and Dopson[1] described many of these mechanisms, which mainly involve maintaining a pH gradient across the cellular membrane wall. The wide repertoire of genes for the cell wall of acidophiles is indicative of a complex structure that probably limits the influx of protons into the cell. In the highly impermeable membrane a predominance of potassium-transporting energy-generating enzymes (ATPases) is found in many acidophiles, which possibly creates the positively charged molecules inside the cell inhibiting the influx of protons. Also specific proton pumping systems to remove protons from the cell have been identified although not clearly defined yet.

Organic acids are harmful to acidophiles because they are protonated (and therefore neutrally charged) in a low pH outside the cell and can pass relatively easy into the cell (cyctoplasm) where the proton dissociates at the more pH neutral conditions lowering the pH in the cell. Many acidophiles have the capacity to degrade organic acids and it is interesting to note that the most extreme acidophiles are all heterotrophs.

## Our search for life at low pH

The aim of our project, is to find microorganisms that use carbon disulfide ($CS_2$) as a carbon and energy source, with a high affinity for carbon disulfide, and able to tolerate high acidity levels as extreme as 10% w/v $H_2SO_4$, a concentration at which it is economically viable to reuse the trickling water within certain factories emitting carbon disulfide.

In earlier work from a full-scale biotrickling reactor, treating gas streams that contain carbon disulfide and hydrogen sulfide, a sulfur-oxidizing bacterium was isolated. The bacterium, which will be described elsewhere, was found to oxidize a variety of sulfur compounds including carbon disulfide ($CS_2$), carbonyl sulfide (COS), hydrogen sulfide ($H_2S$), elemental sulfur ($S^0$) and thiosulfate ($S_20_3^{2-}$). In contrast to other Thiobacilli-like organisms which can oxidize carbon disulfide, this organism was found to oxidize sulfur compounds at extreme acidic conditions with pH values below 0. Since the strain also showed degradation activity at neutral pH, the strain can be defined as an extremely acidotolerant sulfur-oxidizing bacterium. Under aerobic conditions these sulfur compounds were completely oxidized to sulfate. Chemostat cultivation experiments have been performed in order to determine the kinetic parameters (molar growth yield, $K_s$ value, maximum oxidation rate, maximum growth rate) of the isolate under various environmental conditions. Fluorescent DNA probes are being designed based on the 16S rDNA gene sequence in order to be able to monitor the presence and changes in mixed microbial communities similar to those in biotrickling filters which might contain these microbes.

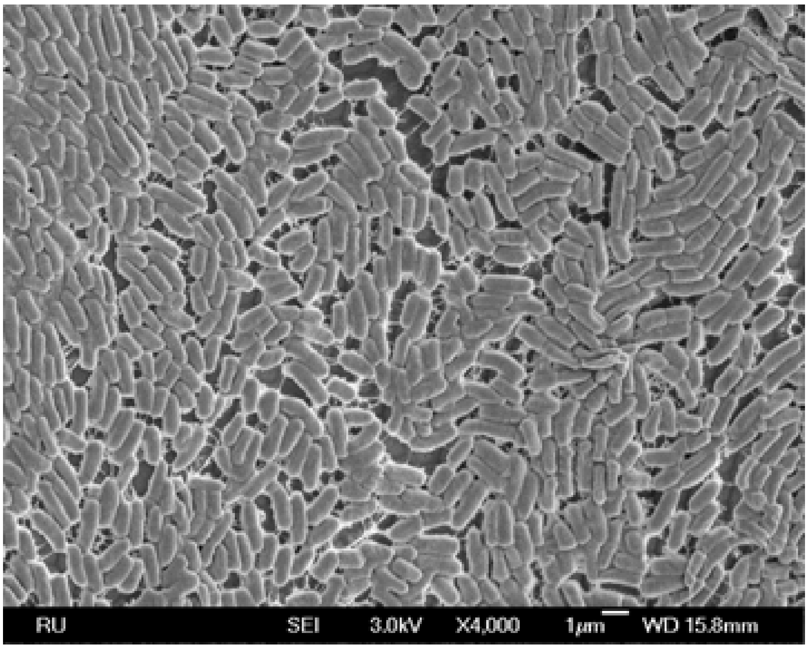


**Fig. 1.** Extremely acidophilic bacteria

In our recent quest to obtain such microorganisms that use carbon disulfide as a carbon and energy source, with a high carbon disulfide affinity, and able to tolerate high acidity levels, we enriched eleven bacterial strains from six different bioreactors treating carbon disulfide containing waste gases from five different industrial plants, and five bacterial strains from environmental samples collected at three highly acidic, sulfur rich sites (pooled hotspring samples, the Solfatara region near Naples and the Solfarata region near Rome). Although we observed substantial phenotypic differences in growth characteristics, 16S DNA analysis showed that all strains were Acidithiobacilli. Several known Acidithiobacillus strains for the ability to convert carbon disulfide were tested but found this is not a trait shared by all Acidithiobacilli. Also the tolerance for acidity levels varied largely with some strains showing very high tolerance for extreme acidic conditions.

## Biological process control at low pH

The low pH process water in a biological wastegas treatment reactor can cause several problems when treating reduced sulfur compounds: 1) a decrease in removal efficiency due to the low pH inhibiting growth and activity of the converting microorganisms, 2) increased potential of clogging of the system by elemental sulfur produced by stressed bacterial cells, 3) an increase in costs due to the large amounts of water required to remove the produced acid during treatment of highly concentrated waste gasses. Extreme acidophilic micro-organisms are able to cope much better with the stress condition of low pH and reduce or eliminate these problems.

In bench-scale reactor experiments we have seen that the biomass growth is reduced when the process conditions of the micro-organisms where more extreme acidic (data not shown here). Sulfuric acid concentration is found to be an important factor in the development of pressure drop over the reactor. Sulfuric acid concentrations of > 3.5-4% (w/w) significantly limited the development of pressure drops over the reactors. At 5% sulfuric acid the pressure drop over the reactor even seems to stabilize. The expected accumulation of elemental sulfur was not observed and consequently did not cause increase in pressure drop. Actual gas retention time determination showed clearly the difference in biofilters gas distribution at different acid conditions as effect on the biomass accumulation. The reduced biomass accumulation at the more extreme acidic conditions can be explained by the reduced biomass yield when the bacteria grow under more extreme stressful conditions. An alternative explanation for the effect of sulfuric acid on pressure drop development could be the inhibition of the growth of other secondary micro-organisms that grow in the biofilm of the reduced sulfur compound degrading micro-organisms. The lower biomass growth at least provides opportunities to reduce the risk of extreme biomass growth in a bioreactor treating high loadings.

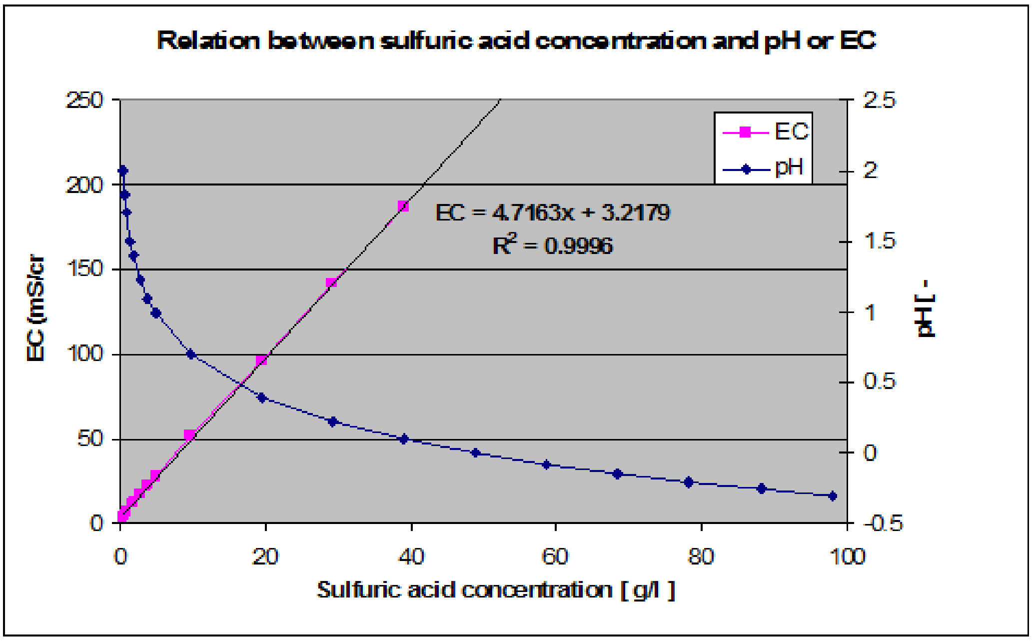


**Fig. 2.** Relationship between acidity, pH and electrical conductivity (EC)

To maintain the most optimal process condition in a bioreactor, control of microbiological process parameters is essential. The pH is especially important when extreme acidic conditions are maintained using acidophilic bacteria. At extreme low acidity the pH can not be measured accurate and the electro-conductivity (EC) can be used. The EC value increases linear with the acid concentration (see Figure 2) and is high enough to have no influence of other ions like nutrients. Using electrical conductivity as a control parameter has the advantage that frequent calibration is not required and that some EC-meters can even give a direct read-out of sulfuric acid concentration.

## Applications

Biotechnological applications using acidophiles is interesting because they can perform conversion in an extreme environment that can have important advantages.

One of the examples of using extreme acidophilic micro-organisms is the wastegas combined treatment of carbon disulfide hydrogen sulfide. Carbon disulfide emissions contribute to the greenhouse gas inventory, because carbon disulfide with its lifetime of 7-12 days is unstable and forms carbonylsulfide (COS). Carbonylsulfide may be transported to the stratosphere where its oxidation is thought to be a major source for stratospheric sulfur aerosol. This aerosol influences the earth's radiation balance and hence the climate[2].

Traditionally, the industrial emissions of carbon disulfide have been treated by adsorption or incineration. These conventional treatment systems have relatively high operational costs and use substantial amounts of energy. They also require many safety measures for explosion-safe operation and generate waste streams (e.g. $SO_2$ emissions or spent activated carbon). Biological processes for waste gas treatment represent an interesting alternative and have been applied already in different industries[3].

The sulfuric acid that is produced in a bioreactor can be neutralized by caustic in the bioreactor to form $Na_2SO_4$, but will require caustic which makes the process much more expensive. The produced sulfuric acid is a clear liquid, but with a concentration normally too low the applicability for re-use in a plant production process is limited. With the more extreme acidophilic micro-organisms this recycling of sulfuric acid is now more interesting and economically feasible in certain situations.

### References

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**Contact: N.J.R. Kraakman**, *Bioway,PO Box 361, 6710 BJ Ede, The Netherlands/USA,* *b.kraakman@bioway.com*