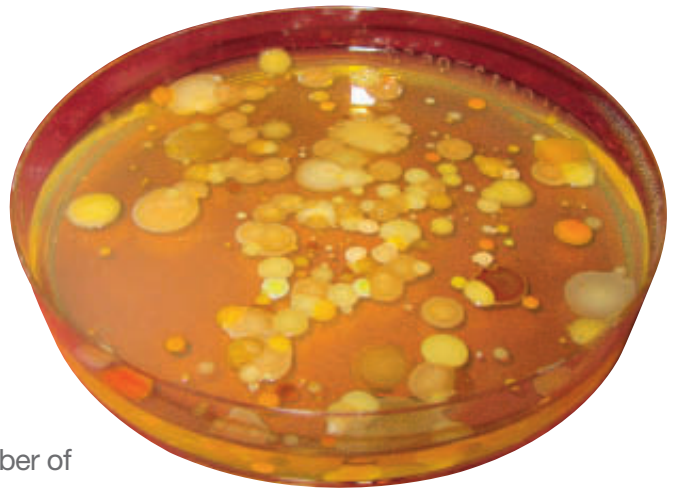


# Microbiological community in biogas systems and evaluation of microbial risks from gas usage

The plans for introducing biogas produced from organic waste to the pipe system for natural gas have raised concerns about the risk of transmitting disease via the gas. To assess this risk, condensate water from gas pipes and gas from different parts of biogas upgrading systems were sampled and cultured for microbial content. The number of microorganisms found in the biogas correspond to the densities in sampled natural gas. Since no pathogens were identified and since the exposure to gas from e. g. cookers and refueling of cars may only result in the inhalation of small volumes of gas, the risk of spreading disease via biogas was judged to be very low.



The variety of MOs found in the condensate water in the gas pipes of the biogas system

Quelle: Vinnerås

**B**iodegradable organic waste (biowaste) is today mainly treated in three different ways in Sweden – by incineration, composting or anaerobic digestion. Additionally, most medium-sized and large wastewater treatment plants treat their sewage sludge anaerobically. Most systems have a mesophilic digestion. For the biogas to have an energy content comparable to that of natural gas, the carbon dioxide has to be removed. A combination of natural gas and biogas can be used in existing infrastructure.

The materials processed in the biogas reactors for biowaste are normally treated at 70 °C for one hour and no non-spore forming pathogens should enter the biogas reactor. However, most of the biogas produced in Sweden comes from sewage sludge treatment and these systems do not contain a pasteurisation step, so pathogens are often found in the sludge (Gantzer et al., 2001). Introducing the biogas produced into systems constructed for natural gas is currently causing a debate about the risks of introducing pathogens to the gas systems. In systems for gas distribution, Zhu et al. (2003) observed, that bacteria could survive and in several cases grow within the biofilm in existing pipes.

To determine, whether there is an actual risk for transmission of disease from the use of biogas, any microorganisms present in the gas have to be enumerated and identified. The separate steps involved in upgrading the raw gas to consumer gas, that can be distributed in a larger network primarily carrying natural gas must also be evaluated, as must sites where people can come in contact with the gas.

The objective of this study was to enumerate and identify the microbiological community in the system for upgrading biogas. This information should be used in combination with a systematic overview of the technical system to assess the risks related to the handling and final usage of the processed gas.

## The biogas systems

Two different biogas systems were investigated. Both consisted of two separate mesophilic digesters (~35 °C) (Fig. 1), one for sewage sludge and one for biowaste. The sewage sludge was not pretreated in order to reduce incoming pathogenic microorganisms before digestion. Incoming material to the reactors treating biowaste was pasteurised at 70 °C for one hour prior to the treatment.

In the systems the gas from both reactors was upgraded in a co-treatment in a pressurised scrubber system. In System 1, the water used in the scrubber was a circulating flow of water, that was regenerated via pressure release in combination with aeration. During the study of the upgraded gas, the gas from the biowaste treatment was led through the back-up system for upgrading, PSA (Pressure Swing Adsorption). In System 2, the water used in the scrubber was continuously fed, not disinfected, treated wastewater (Fig. 1). Additionally, as a reference the content of microorganisms was monitored in natural gas.

## Results

A wide variety of bacteria and a few different fungi were isolated. In general, the total number of microorganisms from the condensate water that were culturable on agar plates was up to 10<sup>5</sup> cfu ml<sup>-1</sup> for bacteria and 10<sup>4</sup> cfu ml<sup>-1</sup> for fungi. The microorganisms identified are presented in Table 1 according to where in the system they were isolated. In the condensate water from System 2 it was also possible to find coliphages, indicating that viruses could be present in the gas. No *Legionella* spp. were found in the condensate water analysed.

When the enumerated microorganisms were divided into bacteria and fungi, it was found that bacteria dominated the flora in the gas. The microorganisms found in the natural gas were in most cases difficult to culture for identification, except for the major classification between fungi and bacteria. The identification indicated the presence of normally occurring environmental bacteria such as Gram-positive rod-shaped bacteria and *Bacillus* spp. (Tab. 1).

### Risk assessment

In order for pathogens that may be present to constitute a true risk for causing infection or disease, it is necessary for individuals to be exposed to the material. The dose required to cause an infection depends on the type of microorganism and the health status of the individual. The microorganisms present in the gas and in the condensate water were screened by sampling, culture and identification. Most of the organisms identified were opportunistic pathogens, which mainly cause infections in immuno-compromised individuals. Some of the bacteria identified (e. g. *E. coli*) occur naturally in the human intestine and can thus be said to be indicators of faecal contamination. The fact that enteric pathogens were not identified is due either to a very low concentration compared to other organisms, rendering them impossible to culture on the substrates, or to them not being present at all. The drying of the gas will significantly reduce the content of microorganisms present, and is another factor contributing to a low probability of pathogens in the upgraded gas.

Exposure to gas could occur through leakages or smaller accidental releases during maintenance of the system. It is also estimated that up to 5 cm<sup>3</sup> of gas may be released to the surroundings during each fill of vehicle tanks. Similar volumes may reach individuals when igniting gas cookers. The other flow is the condensate water and the scrubber water, but both are handled in a closed system and exposure is only possible during maintenance or other types of service on the technical equipment. As stated above, no actual pathogens were isolated from the system and the specific doses that individuals might be exposed to can therefore not be estimated. For the opportunistic pathogens identified, there are no relations between exposure/dose and probability of infection established.

Since most of the microorganisms isolated were opportunistic pathogens, it can be concluded that normally healthy individuals would not be at significant risk even if they

happened to inhale gas or ingest condensate water. The number of viruses to be expected in the gas and condensate water system is low, as the number of organisms that can be introduced is low and no growth can occur in the system.

### Discussion

No actual pathogens were found in the biogas or condensate water from the biogas piping system. However, several opportu-

nistic pathogens were found in the system, together with microorganisms from groups in which pathogens are included, e. g. *Enterobacteriaceae*.

In the raw gas, produced both from biowaste digestion and sewage sludge digestion, in System 1 it was possible to detect 4-65 cfu m<sup>-3</sup>, mainly bacteria but also fungi. Intestinal flora was found in condensate water from both systems for digesti-

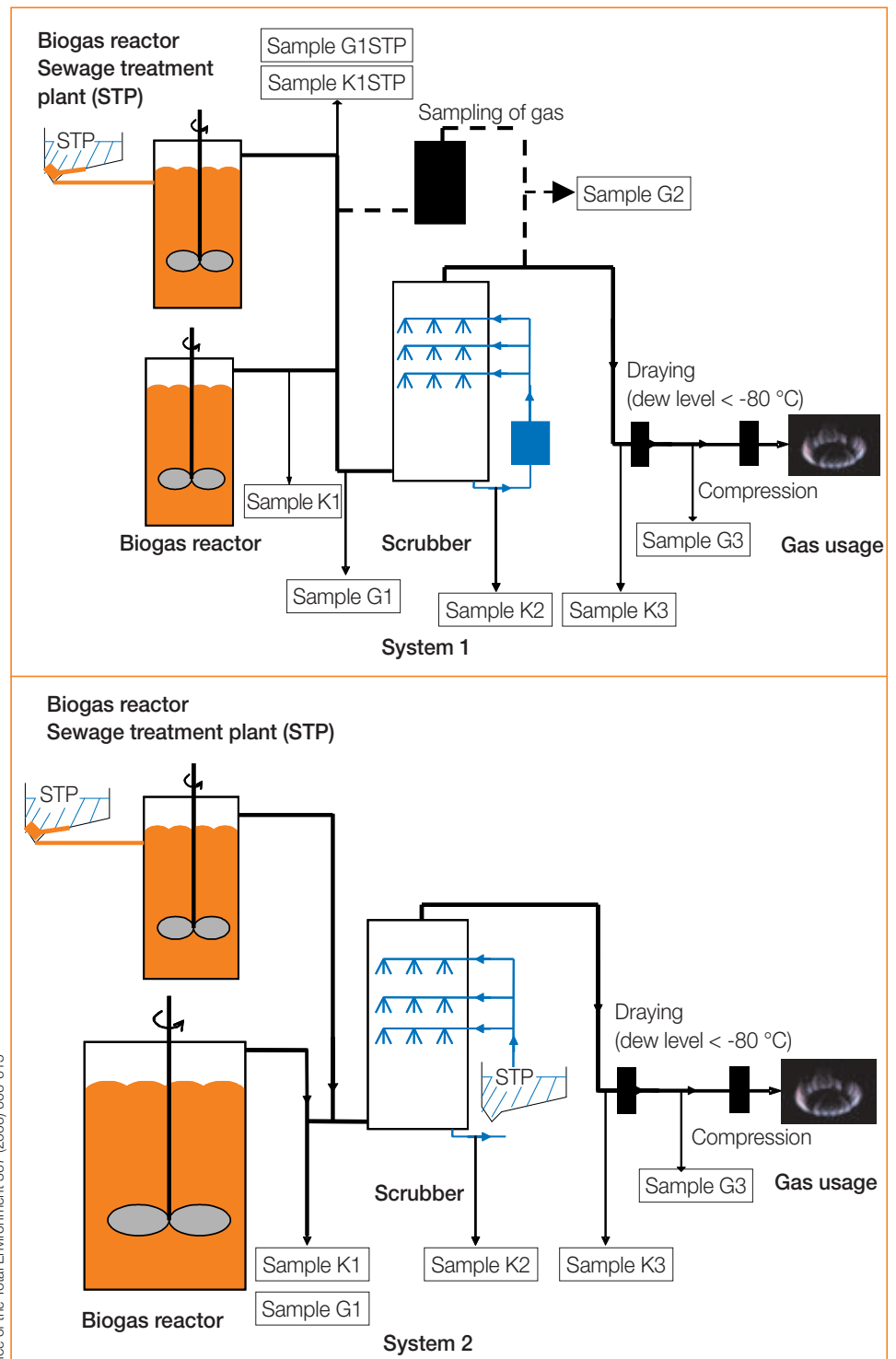


Figure 1: Technical design of the two treatment systems investigated and the sample points for condense (Sample K) and for gas (Sample G)

on of biowaste (Tab. 1), despite thermal treatment (70 °C for one hour) of all incoming material. The only source of faecal contaminants identified was the inoculate used in the reactors. This inoculate came from reactors that treated animal manure without any heat treatment. To ensure that no pathogens enter the biogas reactor, such inoculate needs to be of a high quality and contain no pathogens.

The scrubber water in System 1 showed a high concentration of both, bacteria and

fungi. The circulating water is re-generated by aeration in combination with pressure changes. This treatment puts oxygen, and probably also microorganisms, from the surrounding environment, into the system, allowing aerobic growth of microorganisms in the scrubber tower. Both systems have reported problems with growth on the packing material in the scrubbers.

It was not possible to detect any significant differences in the number of organisms or in the flora between the scrub-

ber and the PSA in System 1. System 2, which used treated wastewater for CO<sub>2</sub> removal, proved to have a higher presence of faecal indicator bacteria than System 1, which circulated the water. The amount of fungi was high in both systems, and it seems that fungi colonise systems that are carbon-based (PSA) or water-based (scrubber).

No significant difference was detected between the concentrations of microorganisms in the upgraded biogas and the bio-

**Table 1: Presentation (name, type, characteristics, pathogenicity) of the microorganisms found in the condensate water collected at location K1-K3 in the treatment plants (Figure 1). G+ = Gram-positive, G- = Gram-negative**

Location	Conc. cfu ml <sup>-1</sup>	Organism	Type of organism	Main characteristics and indication of pathogenicity
K1	–	<i>Clostridium</i> spp. <i>Clostridium perfringens</i>	Anaerobe, G+, rod-shaped, spore-former	Toxin producer, Animal pathogen, Opportunistic pathogen
K1	10 <sup>1</sup>	<i>Aeromonas hydrophila/caviae</i>	G-, rod-shaped	Can cause gastroenteritis
K1	10 <sup>1</sup>	<i>Enterobacteriaceae</i> spp. <i>Klebsiella pneumonia</i>	G-, Oxidase-, rod-shaped bacteria	Pathogens included in the group Opportunistic pathogen, normal intestinal flora
K1	10 <sup>1</sup>	<i>Enterococcus</i> spp.	G+ cocci	Normal intestinal flora
K1	10 <sup>3</sup>	<i>Burkholderia cepacia</i>	G-, rod-shaped	Opportunistic pathogen
K1**	10 <sup>3</sup>	<i>Micrococcus</i> spp.	G+, cocci, Catalase +	Opportunistic pathogen
K2**	10 <sup>2</sup>	<i>Bacillus</i> spp. <i>Bacillus cereus</i>	G+, rod-shaped, endospore-forming,	Potential pathogen, produces enterotoxins
K2	10 <sup>1</sup>	<i>Corynebacterium</i> spp.	G+, rod-shaped	Pathogens included in the group, e.g. causes diphtheria
K2	10 <sup>3</sup>	<i>Pseudomonas</i> spp.	G-, rod-shaped	Opportunistic pathogen
K2	10 <sup>3</sup>	<i>Pseudomonas veronii</i>		
K2	10 <sup>2</sup>	<i>Leucobacter acridicollis</i> <i>Leucobacter komagatae</i> <i>Leucobacter albus</i> <i>Leucobacter chomiireducens</i>	G+, rod-shaped	
K2**	10 <sup>2</sup>	<i>Fusarium</i> spp.	Filamentous fungi	Can cause opportunistic mycoses
K2**	10 <sup>2</sup>	<i>Mucor</i> spp.	Filamentous fungi	Can cause opportunistic mycoses
K3*	10 <sup>0</sup>	Coliphages		
K3	10 <sup>2</sup>	<i>Enterobacteriaceae</i> spp. <i>Chitrobacter freundii</i>	G-, Oxidase-, rod-shaped bacteria	Pathogens included in the group Opportunistic pathogen, normal intestinal flora
K3*	10 <sup>2</sup>	<i>Escherichia coli</i>	G-, Rod-shaped	Can produce verotoxins, normal intestinal flora
K3	10 <sup>1</sup>	<i>Leukobacter</i> spp.	G+, rod-shaped	
K3	10 <sup>3</sup>	<i>Alcaligenes xylos-oxidans</i>	G-, rod-shaped	Opportunistic pathogen
K3	10 <sup>3</sup>	<i>Streptococcus</i> spp.	G+, cocci	Opportunistic pathogen
K3**	10 <sup>3</sup>	<i>Micrococcus</i> spp.	G+, cocci, Catalase +	Opportunistic pathogen
K3	10 <sup>3</sup>	<i>Pseudomonas veronii</i>	G-, rod shaped	Opportunistic pathogen
K3**	10 <sup>4</sup>	<i>Fusarium</i> spp.	Filamentous fungi	Can cause opportunistic mycoses

\*organisms only identified in System 2.

\*\*organisms also found in the gas

gas before upgrading. This indicates that if no active treatment is used for removal of microorganisms, one can expect gas systems to contain a microbial load of approximately 10-100 cfu m<sup>-3</sup>. The main factor to take into account is the risk of introducing pathogens to the system. Pathogens can enter the biogas via the scrubbers, especially the type that uses wastewater. The second source of pathogens is at the sewage treatment plant, if no sanitisation of the sludge is carried out prior to the digestion. The final source of pathogens to the biogas is the inoculation when initially starting the digester. The studies on System 1 proved that even if no *Enterobacteriaceae* enter the system with the raw material, these organisms are constantly found in the outgoing sludge and in the condensate water from the raw gas.

In comparison with other risks related to biogas systems, the risk when using the upgraded biogas is probably insignificant. The risks from coming in contact with raw gas or condensate water in the plant are higher, since at that time these substances have been subjected to a lower degree of treatment than the upgraded gas. However, the system is generally closed and, according to professionals, workers are seldom exposed. The majority of the microorganisms in the digester remain in the digested residue rather than being transported with the gas. In the present study, it was concluded that the wastewater in the scrubber and the biogas produced from sewage sludge would constitute a greater risk of contributing enteric pathogens than the biogas produced from biowaste.

Upgrading the gas to car fuel standard would significantly reduce any microorganisms present as the gas is compressed to

200 bar and filtered through a 1 µm particle filter. By having a general 1 µm particle filter when using the biogas or introducing the biogas into larger gas systems, it would be possible to ensure no passage of the majority of fungi and non-spore forming bacteria.

The advisability of introducing biogas to piping systems constructed for natural gas has been questioned, partly due to hygiene issues. As described above, natural gas was analysed for comparison and was also found to contain spore-forming bacteria such as *Bacillus* spp. and the densities of microorganisms found did not differ much from what was found in the upgraded biogas. Theoretically, the biogas system could contribute pathogens to the system. However, from the results obtained in the present study and from the systematic risk assessment, the indications are that this is quite unlikely.

### Conclusion

In conclusion, risks for disease transmission from processed (upgraded and dried) biogas can be judged as being low. By using the standard filter (1 µm) used for particle removal when using gas as car fuel the risk would be even lower. The possible exposure of personnel is probably the most significant risk. However, this group could be easily identified and informed about potential risks and how they can be managed. The risk of inhaling pathogens when using gas is overshadowed by the risk of gas intoxication and explosions or similar, since these effects would probably occur before a dose of pathogens high enough to cause an infection had been ingested. The biogas produced in the systems analysed is therefore considered safe to use even in kitchen coo-

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