PROSPECTUS

BioGAS+ Research Sample. 200 ml Flask of a \( \text{Fe}_3\text{O}_4 \) colloidal solution 10 mg/ml dispersed in a TMAOH 10 mM water solution.

What it is? BioGAS+ is the first ready to use additive based on safe and sustainable engineered iron based nanoparticles (NPs) directed to the optimization of anaerobic digestion processes which increases the production of Biogas and concentration of \( \text{CH}_4 \).

Applied Nanoparticles offers the trace element supplement BioGAS+, a patented and registered nanotechnology-based innovation that obtains the highest ever-reported improvement of biogas production (200\%)\(^1\).

How it works? BioGAS+ contributes directly to the metabolism of microbes, with the doses of bioavailable iron that they need at each moment, for any microbe and for any feedstock. The mix (\( \text{Fe}^{2+/3+} \)) iron oxide in its mineral form is rather inert in the working conditions but can provide bioavailable \( \text{Fe}^{2+} \) as a response to the electro-bio-mineral redox reactions and the expression of siderophore precursor genes. This is a paradigm shift in the prevalent solutions for Biogas Production optimization.

Instructions for use.

Concentration: 10 mg/ml.

Recommended dose: 0,1\% (w/w) vs VOC\(^2\) of waste to treat.

The provided amount is enough to treat 40 Liters of waste water at the 5\% (w/w) of VOCs. Good results have also been observed with lower (0,01\% vs VOC in w/w) doses.

Just add it up to your digester at any moment, for example along with your periodic fresh waste feeding.

For continuous digesters, we recommend an initial dose for the total digester volume and a recall daily dose proportional to the entrant waste.

BioGAS+ has shown different levels of performance which depend on the triad Nanoparticle-Microbiota-Organic Waste, the presence of sulphur and other chemical interferences, the different archaea in the different consortiums, the acclimatization time, etc.

In our original studies we employed doses of the 10\% of the VOCs following the standardized german DIN-38414 Biomethane Production Test with cellulose and acclimatized inocula from an urban WWTP. Increments up to 200\% of methane production were obtained. Further we observed that only a tiny fraction of our product was actually employed, so we kept decreasing the doses down to 0,01\% while still observing production increments up to 30\% in methane\(^3\).

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2 Volatile Organic Compounds.
3 In a lab/pilot continuous digester at 37°C digesting sheep manure with 21 days resident time.
SHAKE VIGOROUSLY BEFORE USE. Materials denser than water materials tend to sink. Nanocrystals subjected to both Archimedes laws and Brownian Dispersion remain in solution but they may slowly sediment by entropic forces. This is reversible and the solution can be homogeneously recovered by hand shaking before use.

**Storage and Disposal.**

Please keep it closed (it is sold preserved under N₂ atmosphere) and use within 30 days once open. Otherwise we recommend aliquoting it in recipients without head-space to avoid oxidation of BioGAS+. Oxidation of BioGAS+ may result in a change of color of the solution (towards reddish-brown) and slower dissolution rate of the NPs, decreasing the effective released dose.

The product is safe and can be disposed as iron waste. If treated with a mild acidic solution in laboratory conditions it will be transformed in FeCl₃. The product pH is slightly basic and the solution weakly buffered, therefore it should not pose any risk to the environment.

**Safety.**

We consider the safety of our product during storage, use and disposal. Regarding the iron oxide nanoparticles, the product may be present as the original nanoparticles (BG+MSDS1), as microscopic iron oxide particles (aggregates) (BG+MSDS2) and as the derived iron Fe²⁺ (BG+MSDS3) and Fe³⁺ (BG+MSDS4) ions. Please refer to the provided BG+MSDS links. Additionally, refer to the report on the biological interactions of Fe₃O₄ nanocrystals. Regarding the dispersing solution, our TMAOH 10 mM has a pH of 11, therefore follow the usual precautions as when dealing with slightly basic solution (BG+MSDS5).

SAMPLE INTENDED FOR RESEARCH PURPOSES.

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<sup>1</sup> Report on Safety and Toxicity of Iron Oxide Nanoparticles