APPLIED NANOPARTICLES

Monthly Anaerobic Digestion Newsletter

July 2017 compilation and overview of cutting-edge biogas industry news

As we take the last steps from the laboratory to the market and putting our solution in context, Applied Nanoparticles publishes BioGAS+ Newsletter, a compilation and overview of cutting-edge anaerobic digestion industry news in the context of global energy transition and new multipolar world. You can access and download the newsletter at https://www.biogasplus.info/biogasplus-newsletter/ This is a special issue devoted to the release of the beta version of BioGAS+

The time has come! Inaugurating the nanomicrobiology era

Applied Nanoparticles announces availability of BioGAS+ (beta version)

Unique trace element additive for the optimisation of anaerobic digestion

July 14, 2017[0]. In a new step from laboratory to market, with the purpose of making nanotechnology real, Applied Nanoparticles SL announces the availability of their patented, registered and unique product, BioGAS+, that obtains the highest ever-reported improvement of biogas production, among many other differential advantages[1].”

* TAXES AND SHIPMENT NOT INCLUDED
The orders of product may be made since September (all details will be explained in BioGAS+ website: https://biogasplus.info), they will enjoy of a special beta version price and will be served in a *first in, first out* basis.

The trace element additive BioGAS+ contributes directly to the metabolism of microbiota.

Based on innocuous iron oxide nanocrystals that dissolves at the demand of archaea in order to boost their metabolic activity, increasing thus biomethane production and keeping a healthy consortium stabilizing your process. The increased biomethane production is concomitant with a reduction of the digestate volume and its chemical potential (reactivity), and the final digestate is enriched in iron ions, being a better fertilizer. Increased anaerobic digestion also translates into an increased sanitation and process acceleration. Additionally, iron ions precipitate S and P species and attenuates pH variations. Interestingly, BioGAS+ has been observed to promote the degradation of recalcitrant matter and rescue of "sick digesters". The ability of nanoparticles to absorb tensioactives resulting on reducing foams has been also observed. This is a paradigm shift in the prevalent solutions for optimization of biogas production. BioGAS+ is developed under the principles of Responsible Research and Innovation (RRI)[2]. The technology is safe and environmentally friendly, contributing to sustainable energy production and waste management. It produces not only greener energy but also greener digestate (ready to be compost). **Anaerobic Digestion will never be the same**.

Recently, there has been a general turn in the European Union towards green energies (the one that started with the "Energiewende"). Governments are pushing for renewable energy infrastructure and production. By 2020, 20% of the energy consumed in the EU will be renewable (2009/28/CE).

When we first observed the effects of Fe_3O_4 nanoparticles (NPs) in anaerobic digesters we did not really realize what was happening. We were studying the inhibition potential of inorganic NPs and their toxicity to anaerobic consortia in wastewater treatment plants (WWTPs) in the framework of a research project of the Spanish Ministerio de Medio Ambiente y Medio Rural y Marino, where biogas production/inhibition was an endpoint to test NP toxicity. Our results published in Journal of Hazardous Materials[3] and Small[1] showed that non silver or gold or TiO_2 NPs had any negative effect, CeO_2 NPs produced inhibition of biogas production likely due to the Ce^{3+} ions, and an increase of production in the case of Fe_3O_4. Indeed, an external colleague was who pointed it out saying: "*Hey guys, you are triplicating biogas production! This is way above anything I have ever seen!*"

This let us confused for a while. It could be an artifact, but normally, when something goes experimentally wrong, you get less biogas, not more... Then we started reproducing the experiment, developing models and adding controls until the phenomena was slightly understood (so we could take a direction to evolve): **Dosing at the Nanoscale**.

Dosing at the nanoscale is a principle translated from current pharmacology and drug delivery technology. For example, to treat ferropenic anemia in some conditions, patients are injected large amounts of pre-biotic (mineral) iron (amounts that in the ionic form would be toxic) that slowly transform inside the body into Fe^{2+} biologically beneficial ions. BioGAS+ is an analogue approach to treat the endemic ferropenic anemia of archaea.

Once the trail was drawn, things speeded up: getting a patent, looking for and obtaining funding, scaling up the production of the NPs, scaling up the application of the NPs; and doing it all under safety by design and RRI principles. After the initial academic research we obtained funding from the Bill and Melinda Gates Foundation. A Patent was deposited in Europe by patent application 12707361.7.1352 Ref. P1923EP01/EP2683662 and in the USA by patent application nr. 14/004.646 and publication nr. 2014/0017753. Priority Data: 11/03/2011. We were given a Secretaria General Iberoamericana prize in 2013. Our description of the observed phenomena was accepted for publication in the specialized journal Small[1]. In 2013 the patent was made public. Then we obtained strong support from Repsol Foundation for two years incubation and we succeeded in

industrializing the production of our nanoparticles and moving our biogas production tests from batch to semicontinuous pilots replica of anaerobic industrial digesters.

Today, the world of biogas is big and complex. We need co-developers, indeed. We hope that we will create a community of optimized Anaerobic Digestion where nanoparticles can play many beneficial roles. Therefore, we are selling it at production costs and at amounts dimensioned for research purposes.

We will start **accepting orders in September 2017** (all details will be explained in BioGAS+ website: https://biogasplus.info), they will enjoy of a special beta version price and it will be served in a *first in, first out* basis.

Recently, Víctor Puntes, nanoscientist and founder of Applied Nanoparticles said:

> *"BioGAS+ is the result of intensive research that started in 2008 under the principles of Responsible Research and Innovation and will be marketed from next summer. 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. BioGAS+ contributes directly to the metabolism of microbes, with the doses of innocuous and bioavailable iron that needs the microbe at each moment. This is a paradigm shift in the prevalent solutions for optimization. The BioGAS+ enhancement lies on nanomaterial specifications, rather than material composition, a field which we dominate. We feel inaugurating the nanomicrobiology era. Anaerobic Digestion will never be the same."*

About Applied Nanoparticles SL

Applied Nanoparticles SL (AppNPs) is a technology-based spin off company derived from the Universitat Autònoma de Barcelona (UAB), the Institut Català de Recerca i Estudis Avançats (ICREA), and the Catalan Institute of Nanoscience and Nanotechnology (ICN2). The company was founded on October 17th 2013. The company's purpose is to research, study and develop nanoparticles and their applications. This includes design, production and characterization of nanoparticles, consultancy activities and dissemination, as well as training and education in nanoscience, nanotechnology, and related areas under principles of Responsible Research and Innovation. AppNPs core project is the commercial exploitation of a EU-US patent, BioGAS+. AppNPs ultimate aspiration when developing BioGAS+ is to transform waste into appealing raw materials in an efficient and sustainable way while contributing to key policy objectives, such as climate change targets, energy and food security, resource efficiency, improved air quality, the bioeconomy, bioenergy, the prevention of contamination and develop circular economy. You can find out more information from our website: https://www.appliednanoparticles.eu/

[0] We have chosen this date, 14 July, as our humble homage and recognition to the Age of Enlightenment.

[1] Programmed iron oxide nanoparticles disintegration in anaerobic digesters boosts biogas production. Small, 2014, 10, 2801-2810.

[2] https://innovation-compass.eu/wp-content/uploads/2017/04/AppNPs-Final.pdf

[3] Effect of cerium dioxide, titanium dioxide, silver, and gold nanoparticles on the activity of microbial communities intended in wastewater treatment Journal of Hazardous Materials 2012, 199, 64– 72.

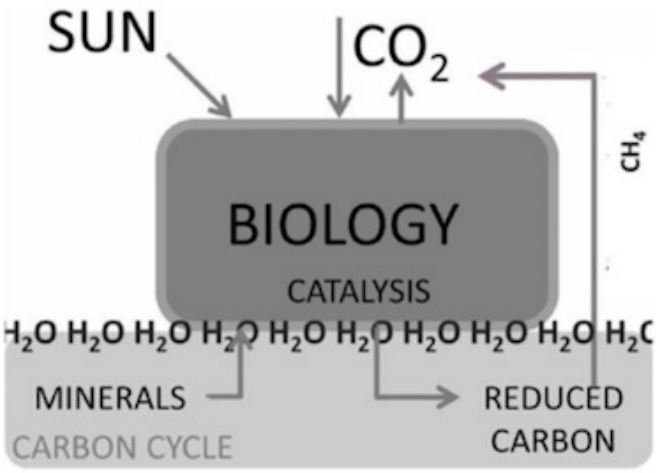
The Carbon Cycle. Methanisation and Iron Oxide Nanoparticles (Extracted from *Starting Up under RRI principles* by Marti Busquets-Fité, Eudald Casals, Ignasi Gispert, Josep Saldaña and Victor Puntes. Applied Nanoparticles). The majority of the energy around us comes directly or indirectly from the sun. The small remaining extra portion comes from heat at the centre of the planet that is slowly cooling, from the gravitational pendulum of the moon and the seas, and from splitting heavy atoms in nuclear reactors. The rest, like the wind, is produced by the sun, which heats air masses that expand and become lighter displacing cooler ones. The wind also moves the waves. There is also the sun energy stored in chemical bonds by biology. This is why wood burns. And coal is fossilized wood. Photosynthesis is a very interesting way to store energy. Trees are made of condensed air by converting CO_2 into organic molecules and carbon based materials. Only a part of the water and little amounts of essential minerals are taken by the roots and come up to the leaves by capillarity. The roots are also made of air, from the CO_2 in air that is reduced by the sun via the photosynthesis. Thus, when the tree and the trunk are burned, the heat generated is a portion of the energy they took from the sun to build themselves. A portion, because there is always a loss of energy in any transformation, dissipated in form of entropy.

A chemical reduction reaction that begins with CO_2 in the atmosphere, accumulates it in the form of hydrocarbons and, progressively, in a multistep manner, evolves towards carbon, petroleum and shale gas. CO2 is taken from the atmosphere, digested, transformed into more complex molecules and oxygen released. Ironically, the end of life in the planet will occur when no more CO_2 is available, when all the Carbon has been buried in solid and liquid forms into the rocks and the ocean depths. “Ironically”, because today the massive extraction of fossil fuels that is taking place is subtly altering the chemical composition of the atmosphere by releasing vast amounts of CO_2 that was previously reduced by photosynthesis, thus going back on geochemical time, with very apparent consequences for climate change.

All organic matter, the biosphere, in its natural reduced state, is immersed in an atmosphere rich in oxygen, and has stored energy. As such, organic waste, pig manure and excrements also store energy. When something stops living, it decomposes. Being alive prevents us from decaying in a few hours. This decomposition ends up returning humidity in the form of water vapour, degraded organic matter, ultimately in the form of CO_2, small parts of other gases, and ashes with nitrates, phosphates and small amounts of other inorganic matter. Interestingly, if this process occurs in conditions where there is a low oxygen concentration, such as the naturally occurring underwater, underground or in man-made closed recipients; the organic matter is degraded into methane, CH_4. This is because a fraction of this organic matter, in the form of archaea bacteria, can breathe the oxygen bound in organic molecules and release methane. This molecule can easily be stored and transported for its posterior burning into CO_2, releasing thus all the energy contained in its four chemical bonds.

This process of transformation of organic matter into methane, or methanization, is not a spontaneous chemical process. It is produced by consortiums of specialized archaic bacteria. These bacteria were among the first inhabitants of our planet. It is said that in that free- oxygen-less world then, life forms incorporated carbon into their organic matter by capturing CO_2 through photosynthesis. In the process they released 2 atoms of oxygen that progressively accumulated as a toxic waste in the atmosphere. Oxygen is of course very reactive, and it was very toxic back then. This is why we can still use hydrogen peroxide, H_2O_2, as a disinfectant. It burns things. And it was this waste from life, from initial metabolism, that caused the first massive extinction 2.400 million years ago in the Precambrian era. Not all Precambrian forms of life disappeared. Some stayed alive in places without a good oxygen supply, at the bottom of wells, between rocks, inside of living things (the concentration of free oxygen inside the body is very low; it’s all transported by haemoglobin), and they produce methane. This is why sewers explode when there is an accidental spark. These bacteria are everywhere, proliferating whenever they have the opportunity to access

organic matter in the absence of oxygen – excrement, under skin or corpse. When exposed to oxygen, many die while some form spores to wait for more optimal conditions for their biochemical living.



Carbon Cycle. Note that part of the reduced carbon is transformed into CH_4 which is constantly emitted to the atmosphere where it will be slowly oxydized to CO_2. Before that, in the form of CH_4, it produces about 20 times more green house effect than CO_2

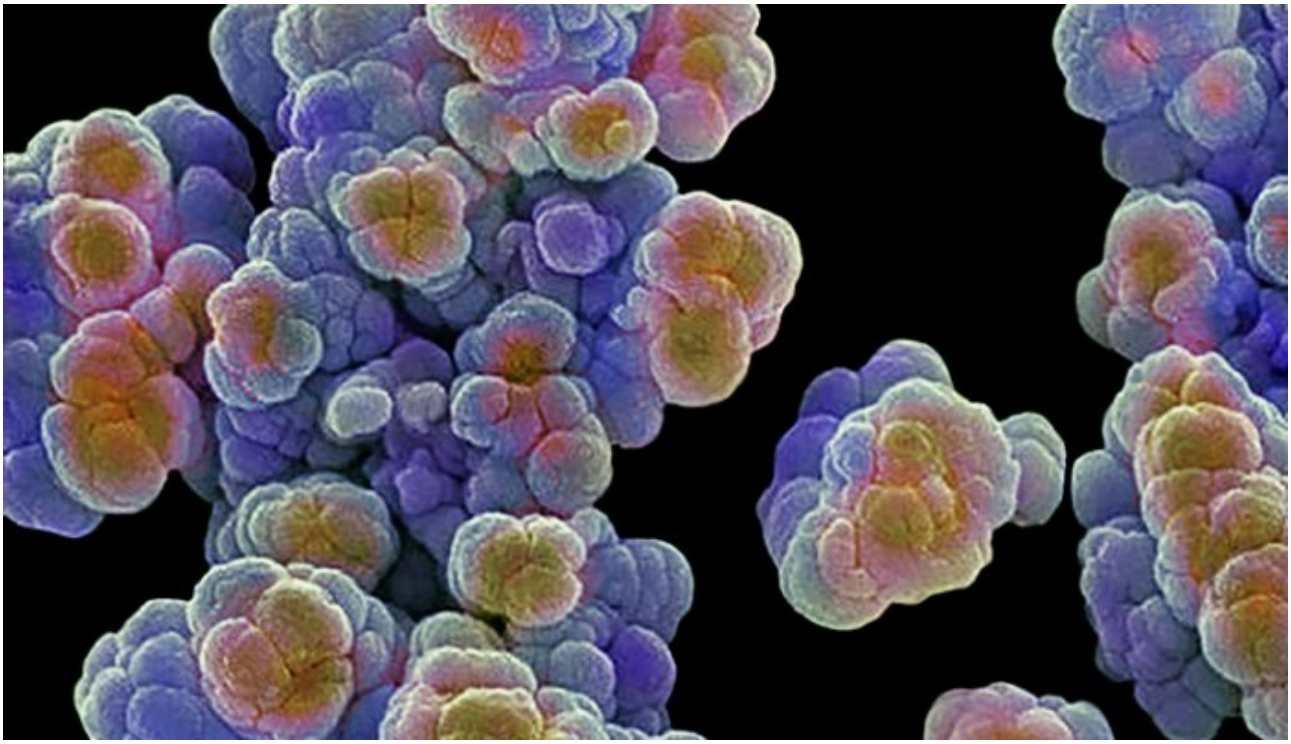
Both anaerobic and aerobic bacteria need iron for their functioning, like animals, plants and fungi. In fact, all life forms base part of their metabolism on the oxidative reduction of iron ions between valence states +2 and +3. In physiological conditions, iron can easily afford to donate or take an electron. Taking and giving away electrons is the essence of (bio) chemistry. Normally, bacteria does not store iron, as mammals do with ferritin, therefore, they need to take it from the environment. In the environment, there is a great abundance of iron in its inorganic form. The planet's core is made of iron and it is the fourth most abundant element in the crust. But it takes an important biochemical effort to transform the iron found in rocks into biologically available, like in blood. For us, eating screws or red soil will never cure anaemia, but microbes can do it, even if they also prefer to take iron already inserted in the biological units. Thus, when bacteria infect an organism, the largest and most immediate genetic expression alteration they experiment has to do with the finding, trapping and use of iron for their proliferation. And it is for this reason that when bacteria are detected by the immune system, one of the first defence actions is to remove iron and sugar available in blood.

Interestingly, in this context, in conditions of anaerobic breakdown, in the absence of oxygen, small doses of mixed iron oxide nanoparticles (NPs) serve as a *catalyst* that stimulates bacteria metabolism and accelerates the production of biogas (a mixture of different gases produced by the breakdown of organic matter in the absence of oxygen, mainly CO_2 and CH_4) up to three times with cellulose as feedstock in laboratory conditions (DIN-38414). Thus, the process that converts organic waste into raw matter for energy production is optimized by simply adding a small dose of iron NPs either to a large waste treatment reactor, a septic tank or a homemade biodigester. This is based on the effects of the presence of essential trace elements in the methanogenesis process, and the optimized dosing when using small unstable NPs that corrode and dissolve as ions provider. In fact, a challenging area of anaerobic digestion research remains largely unchartered with respect to understanding the role of trace metals in enabling biogas production. This major knowledge gap and scientific challenge is a multifaceted problem involving metal chemistry, physical interactions of metal and solids, microbiology and technology optimisation. **Read More**

Applied Nanoparticles: Starting up under Responsible Research and Innovation principles by Marti Busquets-Fité, Eudald Casals, Ignasi Gispert, Josep Saldaña and Victor Puntes; Applied Nanoparticles. Abstract. Applied Nanoparticles S.L. (AppNPs) is a spin-off of the Catalan Institute of Nanoscience and Nanotechnology (ICN2), the University Autònoma of Barcelona (UAB) and the Institut Català de Recerca i Estudis Avançats (ICREA). Among its co-founders are scientists from these institutions, and experts on Responsible Research and Innovation (RRI), e-communication and business development and technology transfer. AppNPs has the office address in Barcelona. The main current objective of AppNPs is the commercial exploitation of the patent application "biogas production" (BioGAS+), in the U.S. and Europe, consisting on the use of iron oxide nanoparticles as additives to optimise the production of biogas by feeding with essential iron the involved bacterial consortia responsible for that. AppNPs also develops projects on the production, characterisation and commercialization of model nanoparticles, as well as consulting work related to other possible industrial uses of inorganic nanoparticles. **AppNPs business is based on the principles of Responsible Innovation, focusing on the process design of nanoparticles, low energy consumption, low toxicity, waste minimization and reduction of emissions**. **Read More**

context:

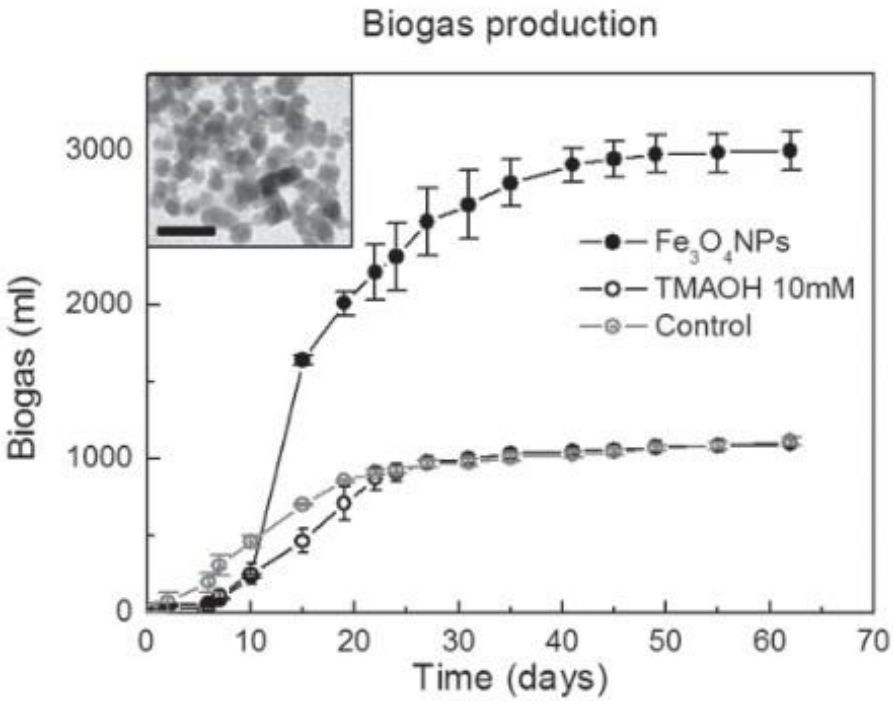
March, 2017. "*They made it! Inspiring testimonial from a company which integrated Responsible Research and Innovation principles in their activity: Applied Nanoparticles SL,*" 1st winner of the COMPASS case studies. "RRI Case Study. Applied Nanoparticles SL: Spinning off under Responsible Research and Innovation (RRI) principles."



The process of transformation of organic matter into methane, or methanization, is not a spontaneous chemical process. It is produced by consortiums of specialized archaic bacteria. These bacteria were among the first inhabitants of our planet

Report on Safety and Toxicity of Iron Oxide Nanoparticles. BioGAS + by Applied Nanoparticles , 8th February 2016. Summary: Superparamagnetic iron oxide nanoparticles are the only clinically approved metal oxide nanoparticles. Given that iron oxides magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3) occur naturally as nano-sized crystals in the Earth's crust, it would seem that

there is no intrinsic risk associated with these nanoparticles. Magnetite nanoparticles have attracted much attention not only because of their superparamagnetic properties but also because they have been shown to have low toxicity in the human body. Currently, magnetite nanoparticles are used in a variety of biomedical applications, for example, magnetic resonance imaging, targeted delivery of drugs or genes, targeted destruction of tumor tissue through hyperthermia, magnetic transfections, iron detection, chelation therapy, anemia treatment and tissue engineering. Several studies have examined the toxicity potential of several different types of magnetite nanoparticles with a range of surface coatings and have generally found low or no toxicity associated with these nanoparticles until high exposure levels (>100 mg/ml). The toxicity was also found to be dependent on various factors such as type of surface coating or its breakdown products, tail length, chemical composition of cell-medium, oxidation state and protein interaction. In conclusion, many studies have shown that magnetite nanoparticles with a range of surface coatings have low or no toxicity except at very high levels of exposure. **Read More**



A novel concept of dosing iron ions using Fe3O4 engineered nanoparticles is used to improve biogas production in anaerobic digestion processes

Differential advantages of BioGAS+ by Applied Nanoparticles. "Biogas+ is the first ready to use additive based on safe and sustainable engineered iron based nanoparticles directed to the optimization of anaerobic digestion processes which increases the production of biogas and concentration of CH_4. Thus, the process that converts organic waste into raw matter for energy production is optimized by simply adding a small dose of iron based nanoparticles to either a large waste treatment reactor, a septic tank, or a homemade biodigester, you just spike the nanoparticles solution onto it. **BiogasPLUS is a disruptive technology because it obtains the highest ever-reported improvement of biogas production**: triples (200% increase of production) the biogas yield with cellulose as feedstock in laboratory conditions and obtains over a 30% methane ratio increase in real industrial settings, with real feedstock and with optimal concentrations below the 1% (with respect to the Volatile Solids). Such a methane production increase is far above any known technology aimed at increasing biogas production: many existing technologies approach this problem (i.e. pre-treatment of the biomass, thermalization of the waste, combination of feedstock

and inoculums) but only obtain modest production increases. Moreover, many tend to be costly to implement since they usually require structural changes in the biogas production process. **The unprecedented methane production increase is the most appealing advantage of BioGAS+, but it also offers additional differential advantages, including**:

1. Increase in both biogas and biomethane production.
2. Improving biomass to biomethane conversion effciency.
3. Better biogas composition (higher methane share).
4. Reduction of the digestate fraction (more biogas means more digestion).
5. Higher waste degradation (the digestate is less, and less reactive).
6. Increase digestion process stability (more reproducible).
7. Acceleration of the digestion process. Reduction in retention/residential time.
8. Proven to reduce H_2S levels (precipitated in the form of pyrite).
9. Reduction on the amount of foam produced (small particles trap detergents decreasing foam).
10. Enrichment of the residual material (digestate) with iron ions to obtain by-products with increased economic value such as high quality fertilizers.
11. Solution to inhibitory substances. Rescue of digesters with problems.
12. Simple additive that can be just spiked onto the incoming sewage (it does not require any change in the biogas plant industrial process).
13. It does not require pre-treatment of the substrate/feedstock or maintenance to preserve the microorganisms.
14. Enlargement of biomass feedstock (oil, fat, meat) as it has been proved very suitable for "diffcult to digest" (recalcitrant) feedstock.
15. Enlargement of biomass feedstock (low energy waste) due to the increased biogas/methane production.
16. Can be used with any kind of anaerobic digester, the process is fully scalable.
17. Reduce AD plant energy consumption.
18. Minimize undesirable side effects in biogas plants such as the odours associated to HS and NH3, thus reducing the cost of associated conditioning measures.
19. Precipitation (recovery) of phosphorus (in the form of ferric and ferrous phosphate).
20. Disinfection of pathogens and multi-resistant bacteria.

context:

July 20, 2017. **Applied Nanoparticles in The Biomethane Map**. The Biomethane Map identifies different innovative process and technology solutions along the biomethane supply chain

July 12, 2017. **Applied Nanoparticles Receives the Highly Commended Award for Research Project**. Back for the sixth year running, the 2017 AD & Biogas Industry Awards will showcase the very best plants, successes and people across the AD industry, both in the UK and around the world.

June 2, 2017. **Milestones of BiogasPLUS by Applied Nanoparticles**. "BiogasPLUS is inaugurating the era of nano-micro-bio-technology."