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Stimulation of aerobic and anaerobic biological processes by ultrasound

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Abstract:

Operators of wastewater treatment plants first of all are responsible for the required good quality of the effluent before it is discharged to the environment. Today attention is being shifted more and more to optimize the operation of treatment plants not only to reduce energy consumption or other costs in an economic sense but with regard to ecological aspects like reduction of greenhouse gas emissions. So the challenge arises to improve the well established biological treatment processes targeting these goals without decreasing the quality of the effluents. First full-scale applications of high power ultrasound appeared more than ten years ago in Germany for the pre-treatment of waste activated sludge prior to anaerobic stabilization. Today about 80 treatment plants worldwide are equipped with this technology and provide confirmation on the success of this approach. Research is still carried out to better understand how ultrasound manifestations can impact also on aerobic biomass. This article reports on fundamental research as well as full-scale experience for the following applications:

- Improving N removal
- Reducing excess sludge
- Combating filamentous microorganisms

INTRODUCTION

Research on ultrasound technology in environmental engineering started about the middle of the nineties last century and was mainly driven by work in Germany. Early it was recognized that a big potential of that innovative technology would be in improving the traditional anaerobic stabilization of organic sludge produced during the biological wastewater treatment process. This process was applied already in practice for more than half a century with no major improvement and no serious attempt to overcome its technical limitations. The idea was to overcome the rate limiting hydrolysis step of the anaerobic sludge stabilization by ultrasound, which disintegrates sludge cells and subsequently intensifies the anaerobic degradation process, eventually resulting in more biogas and less residual sludge. The first full-scale ultrasound installations appeared in Germany after the turn of the millennium. Today plants equipped with this new technology can be found in various European countries including France, Spain, Ireland, Switzerland, the Netherlands, Denmark, Poland, Italy and Austria.

It has been proven that low-frequency ultrasound (below 100 kHz) generates the cavitation necessary to produce the mechanical shear forces associated with sludge disintegration [1]. Combined with high-intensity ultrasound of 25-50 W/cm^2, the cell aggregates as well as single cells are destroyed and enzymatic and intracellular material is released into the medium resulting in a higher degree of substrate bio-availability for the remaining living micro-organisms. In effect the enzymatic-biological hydrolysis, which is the initial and rate-limiting step of the anaerobic food chain, is substituted and catalysed by this mechanical disintegration of the sludge [2].

To illustrate the effect of ultrasonic treatment in full practice we first report briefly on anaerobic stabilization process, viz. two long lasting but differing cases from Bamberg and Meldorf WWTP resp., both Germany. More detailed information was published earlier by Wolff et al. [3] and Herzberg & Houy [4]. These paragraphs are followed by reports about the more recent applications for improved aerobic treatment (N removal and combating bulking sludge).

ENHANCING ANAEROBIC DIGESTION – BAMBERG WWTP

The Bamberg WWTP was designed for 220,000 PE. However, as a result of an improvement and extension of the sewerage system, the load on the plant in 2002 increased up to about 330,000 PE. The plant is equipped with three mesophilic anaerobic digesters to treat a mixture of primary and secondary sludge (WAS). As a consequence of the increased load, more sludge was produced and the HRT in the digesters dropped to just 18 days. The initial plan was to construct a new, fourth digester with a volume of 3,000 m^3. However, the management of the plant decided to test the newly developed Ultrawaves ultrasound technology during a full-scale trial period of four months. After feeding the digesters with ultrasonically treated WAS, the gas production showed a marked increase of almost 30%. The methane content also increased slightly making the biogas a more attractive and energy rich product. The residual VS content in the digested sludge was reduced from 60% to 54%. The desired goal to reach a minimum of 40% VS degradation was not only met, but surpassed.

After the successful trial period in 2002, the Bamberg WWTP management decided to purchase two US reactors (2 x 5 kW) instead of building a new digester thus avoiding the costly undertaking of such a construction. The high-power ultrasound units were installed in August 2004 with the same objective of enhancing VS degradation to a minimum of 40%. In order to achieve this, the system was designed to sonicate at least 30% of the thickened WAS (TWAS) flow before being fed to the digesters. Figure 1(A) is a picture of the two high-power ultrasound reactors which were installed in Bamberg. Figure 1(B) shows the uncovered ultrasound unit. As is clear in both photos, the small footprint of the equipment and the simple way that it can be connected to existing piping and installations make it a very attractive piece of technology.

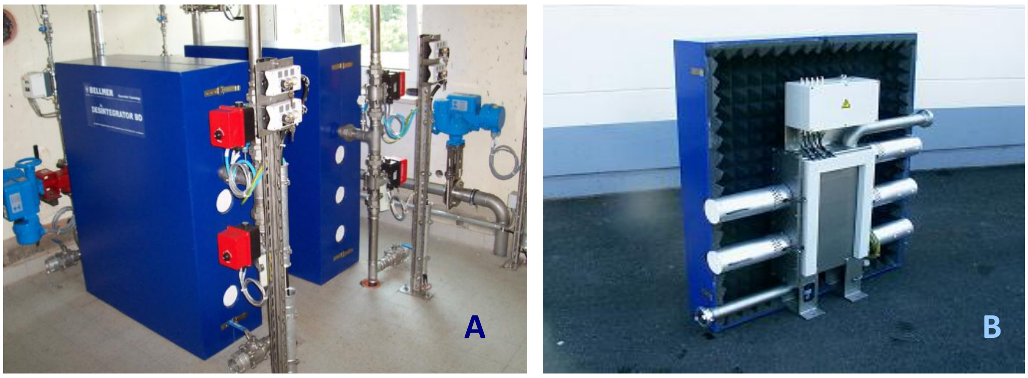

Figure 1: (A) Two high-power ultrasound reactors of Ultrawaves Co. at Bamberg WWTP for the disintegration of waste activated sludge. The two units are equipped with sound protection covers. (B) The sound protection cover is removed from one 5 kW ultrasound unit; inflow lower left, outflow upper right. Five perforated aluminium tubes accommodate mechanical aerators for proper removal of heat from the piezo-ceramic transducers.

Calculating organic matter mass balances at full-scale WWTP is a challenging task. Steady state conditions are never reached. The daily fluctuations as well as less than optimal analytical records make it difficult to objectively assess the performance of the sludge stabilisation system. Even under well controlled pilot-scale conditions, a mass balance on the performance of an anaerobic reactor regarding VS often remains incomplete. Our assessment of the impact of ultrasonic TWAS disintegration on the anaerobic digestion process at Bamberg is based on routinely-collected data sets. No additional information could be collected. As a result, comparison can be made only between the period before (1/2003-12/2004) and after the start of ultrasound application followed by long term recording of the data in order to assess the reliability of the new technology. Figure 2 presents the recorded data regarding digester performance since the beginning of year 2003 up to the end of year 2010.

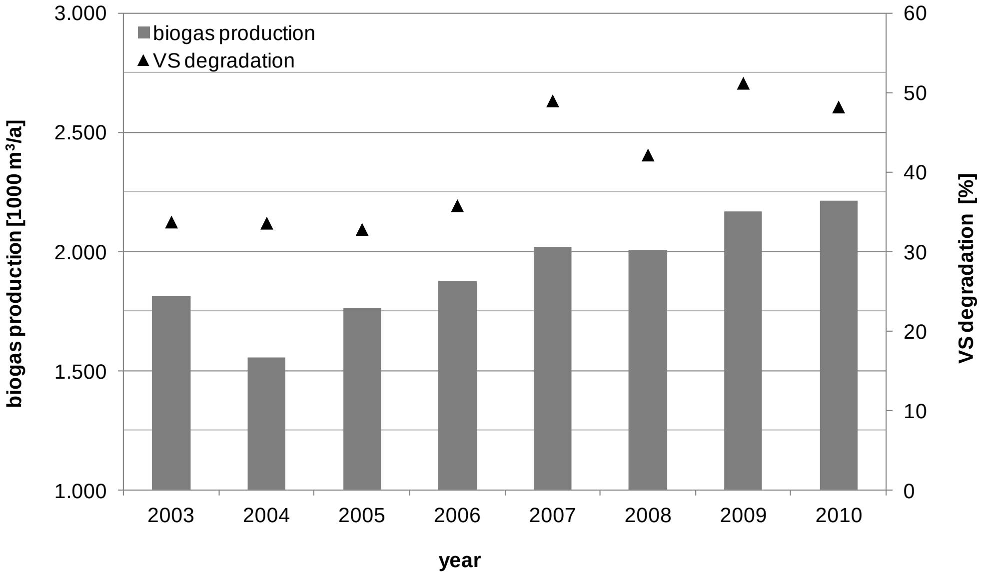

Figure 2: Biogas production and volatile solids degradation on Bamberg WWTP. Two ultrasound units are in operation since August 2004.

While the VS degradation remained constant on a level of about 34% during the years 2003 to 2005 it increased subsequently to about 50% from year 2007 on. The increase of VS degradation followed the increased amount of sonicated waste activate sludge: While during year 2005 roughly 30% of TWAS-flow was treated in the two ultrasound reactors, the share of sonicated TWAS was enhanced stepwise to a constant level of about 80% from year 2007 on.

The increase in the VS destruction implies that more of the organic matter in the sludge was metabolised in the digestion process. This coincides with the increase in gas production (Fig. 2). The total volume of sonicated TWAS in 2008 was 32,310 m^3, corresponding to 88 m^3/d. This represents an ideal volumetric flow for the two installed ultrasound reactors. The ultrasound units are in operation 24 hours per day all year round. The resulting sonication time is 57 seconds.

The annual energy consumed was 70,800 kWh/a, representing only 1.5% of the total energy consumption at the Bamberg WWTP. Based on these data, the calculated average specific energy consumption of the ultrasound units is 2.4 kWh/m^3 TWAS (0.041 kWh/kg DS or 148 kJ/kg DS), a value which is far below data published often from experiences not collected in real life situations at full scale.

COMBATING FILAMENTOUS BACTERIA AND RESTORING ANAEROBIC DIGESTION – MELDORF WWTP

A full-scale ultrasound unit was implemented at Meldorf WWTP (65,000 PE) in February 2005 after a three-months test period. The plant was experiencing problems with foaming in the anaerobic digester as a result of excessive growth of filamentous bacteria (*Microthrix parvicella*) in the waste activated sludge. The purpose of the ultrasound installation was to eliminate the source of the foaming problems and thereby ensuring an undisturbed anaerobic digestion. Ultrasound was applied to 100% of the thickened waste activated sludge (TWAS) flow before to feeding to the two anaerobic digester tanks present at the plant.

A short time after the installation of the ultrasound equipment at Meldorf WWTP, the problems with foaming sludge never appeared again. The operation of the digesters remained stable and undisturbed. The VS were reduced from 60% to 45% in the stabilized sludge. With regard to biogas production, a 30% increase after the ultrasound installation was noted. In addition, the feeding of co-substrates was made possible as a result of the improved stability of the anaerobic digestion process. The plant now was able to accept process liquids from a local food producer, which serves the interests of both parties. Below a schematic is shown on how the ultrasound unit is integrated into the sludge flow patterns.

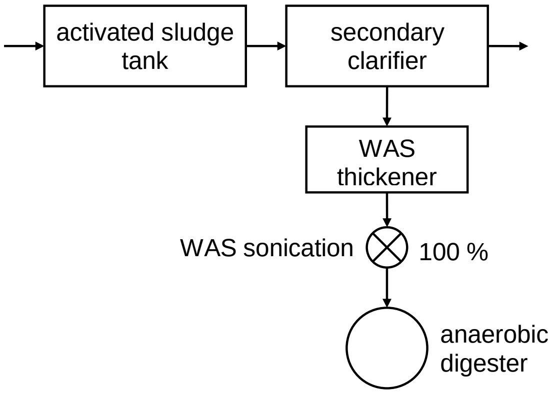

Figure 3: Schematic of wastewater and sludge flows on Meldorf WWTP.

COMBATING FILAMENTOUS BACTERIA AND BULKING SLUDGE – SEEVETAL WWTP

Seevetal WWTP lies about 25 km southwest of Hamburg. It has a capacity of 165,000 PE and biologically treats municipal sewage (activated sludge including nitrogen removal). The sudge age normally turns around 25 days. The plant is operated by the county of Harburg-Land. In winter the formation of bulking sludge on the activated sludge tanks is quite common, which affords additional efforts by the operating staff to remove the sludge layers from the surface of the tanks.

In 2008 we conducted intensive tests with a standard 5 kW ultrasound unit in order to demonstrate the potential of our technology to combat bulking sludge. A detailed presentation of the design and results of this work was already is given in Neis [5]. The interesting situation at Seevetal WWTP is that there are a number of activated sludge tanks, which are operated in parallel. So it was a perfect setting to demonstrate the effect of ultrasound on bulking sludge because we could supply one tank with sonicated return activated sludge as compared to one in parallel which received the normal return sludge. Our idea was to apply ultrasound with rather low energy on a small fraction (approx. 1%) of the return sludge flow, which, of course, is populated by filamentous organisms. By this method we set up a loop of sonicated (and stressed) biomass which permanently is fed to the activated sludge tank. So after a certain period in time the complete biomass has been hit by the ultrasound/cavitation impact. As the results show the high-power Ultrawaves ultrasound unit (25-50 W/cm^2) could selectively eliminate the filamentous bacteria without hampering the treatment efficiency. The bulking sludge disappeared rapidly on the “sonicated” tanks as is shown on the photo in Figure 4.

In the following chapters we will discuss in more detail how high-power ultrasound can impact on aerobic biomass and especially the filamentous species like *Microthrix parvicella.*

Figure 4: Activated sludge tanks of Seevetal WWTP during (A) conventional operation and (B) RAS-sonication.

ENHANCING FULL-SCALE AEROBIC DIGESTION – NITROGEN REMOVAL

Upon ultrasound impact the microorganisms in the activated sludge get stressed and the colonies break apart. In addition, it is believed that the biological processes that proceed in the sludge are stimulated by the release of intercellular material from the damaged cells. Specifically, released enzymes are thought to play a significant role in stimulating various biological processes. The destroyed cellular material itself serves as a non-foreign carbon source for the remaining viable microorganisms in the sludge. In this way we are able to reach an intensified denitrification. We are promoting this innovative approach by sonicating a fraction of the waste activated sludge before returning it to the anoxic sludge tank, Kemalides [6], which entails providing an internal carbon source to the denitrifying bacteria. Though the mechanisms governing the reactions that ultimately result in this improvement are not fully understood yet, we hope to contribute to this end with the following. We start by describing the design and experience of our first full-scale ultrasound application for better nitrogen removal on Bünde WWTP, Westfalia. Later on we will describe the more fundamental research work in our laboratory. It is interesting to report that - despite many articles published on laboratory work with ultrasound - in practice energy consumption of high-intensity (> 25 W/cm^2) ultrasound systems can be held rather low (< 5 kWh/m^3) to stimulate the active biomass in both anaerobic as well as aerobic processes.

Bünde WWTP

One Ultrawaves ultrasound reactor (5 kW) has been in operation at the Bünde WWTP (actual load 54,000 PE) since September 2006. A test period of four months had, by this point, been successfully completed and the decision to proceed with the installation was based on the convincing results obtained during the test period. The nitrogen elimination at the plant occurs via intermittent denitrification. The reason for implementing the ultrasound technology on the plant was that denitrification efficiency was suffering as a result of insufficient carbon source for the denitrifying bacteria. Using sonicated thickened waste activated sludge as an internal carbon source to improve denitrification was seen as a viable option. In total 30% of the daily TWAS stream was sonicated and conveyed back to the activated sludge tank, providing the process with the carbon source needed.

After sonication ceased in July 2006, the concentrations again climbed to the pre-ultrasound levels observed in 2005. Sonicating a partial stream of the thickened waste activated sludge before it goes back to the activated sludge tank provided the carbon source necessary to facilitate the denitrification.

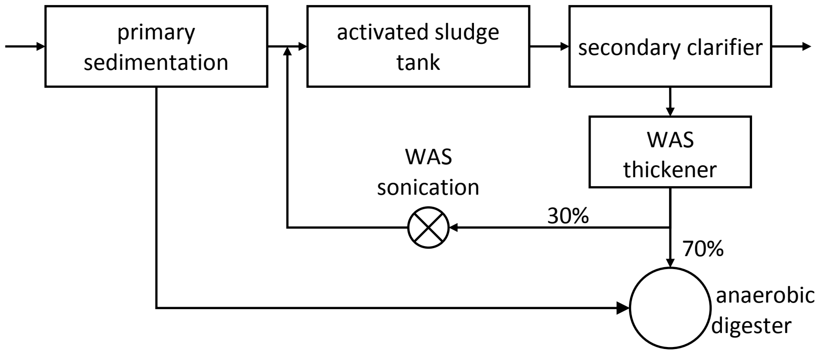

Figure 5: Schematic of wastewater and sludge flows on Bünde WWTP.

Utilizing sonicated TWAS as an internal carbon source for the purpose of improving denitrification yielded several positive effects. First, a significant reduction of nitrogen in circulation at the plant was achieved, which means that denitrification had been improved. In addition, there were secondary effects that contributed to a higher efficiency of the plant operations. Excess sludge was reduced by 25% and foaming and bulking sludge in the activated sludge tank was virtually eliminated.

It is apparent that several positive advantages were reached through the usage of ultrasonically treated sludge. Regarding economics, reimbursement of the investment was immediate as reduced sewage fees associated with sludge disposal and nitrogen concentrations were achieved. Hence, the advantages are visible from an economic perspective as well as on the operational level itself. Adding to economic advantages, it must be mentioned that the plant avoided being forced into purchasing an external carbon source.

The plant was ultimately able to conserve resources, optimize existing process and acquire a higher degree of operational stability through the incorporation of this ultrasound technology.

SONICATION OF AEROBIC BIOMASS - FUNDAMENTALS

So, after all we know that ultrasound can stimulate micro-organism activity in the anaerobic environment. There is no reason not to believe that this is valid too for the aerobic milieu like the activated sludge tank. However, we have to do more research work to better understand what beneficial impacts can be created by ultrasound on activated sludge biomass.
We made a number of investigations on the impact of low energy ultrasound on activated sludge under controlled conditions in the lab either as batch or as continuous systems. Since activated sludge contains numerous and multiple micro-organisms, which are imbedded in a slime-matrix it is not easy to interpret the impact of sonication on the biomass. This is the reason why we studied in addition two bacteria pure cultures, viz. *Microthrix parvicella* and *Pseudomonas aeruginosa. M. parvicella* is a gram positive filamentous organism, which quite often forms clumps. *M. parvicella* often prevails in bulking sludge episodes, [7]. *Pseudomonas aeruginosa* is a gram negative rod like bacteria with a length of 1-2 µm. It is ubiquitous in soil and water bodies. *Pseudomonas* type species have already been identified as denitrifying bacteria in activated sludge, [8].

Materials and methods

Activated sludge samples were taken from Seevetal WWTP. The sludge age was 22 days at the time of sampling. Lightmicroscopic analysis showed a normal floc structure of the activated sludge with average floc size of 100-500 µm. We also observed floc agglomeration as well as many ciliates. Filamentous species were identified as *M. parvicella and Nostocoida limicola* II*,* Eikelboom Typ 0961.

Different microscopic techniques were applied to study the effects of sonication on the activated sludge biomass, viz: light microscope, scanning electron microscope (SEM), transmission electron microscope (TEM).

Cultivation of *M. parvicella* STA 4 was done in R2A liquid media without agar. Tween 40 was added (0,01 g/L) at 20°C, 10 – 12 days, stirring (120 rpm) in dark. *M. parvicella* culture was taken from Bavarian State Office for Water Management.

Cultivation of *P. aeruginosa* AdS was done in Laura Bethani (LB) media at 36°C, 24 h long stirring (140 rpm) in dark. *P. aeruginosa* was isolated as wild strain from a house plumbing system. It was taken from the Institute of Water Resources and Water Supply at Technical University Hamburg-Harburg (TUHH).

Sonication of the biomass

Ultrasound treatment of each biomass sample was performed with an energy input of 2, 5, 10 and 20 Wh/L respectively in a 1-liter beaker. A Branson 450 sonifier (100 Watt) was used with a frequency of 20 kHz. The tip of the oscillating horn during sonication dipped from the top into the biomass suspension, furthermore the sample was thoroughly mixed by a magnetic stirrer. The temperature of the sample was controlled (20 °C) by circulating cooling water through the outer ring of the beaker. The increase in soluble organic carbon (DOC) and total nitrogen (TN) is taken as indicator to assess the impact of ultrasound on the biomass.

Chemical analyses

Total solids (TS) were determined according to German standard DIN 38414 from 10 to 25 mL samples by filtration through washed and dried membrane filters with a pore size of 0,45 µm (Whatman® 42, Ashless). After filtration the samples were dried at 105°C. The dried membrane filters plus solid filter cake were cooled in an exsiccator and weighed afterwards. The filtrate was used to determine DOC and TN by automatic analysis with an infrared absorption detector (Analytik Jena multi N/C 3000).

Microscopical techniques applied

The activated sludge samples were analyzed by using a light microscope (Olympus BX41) at different magnifications. Samples in the native state were examined at magnification of 100. The shape and the structure of flocs were determined. For the recordings a digital camera (Olympus C3040 ZOOM) coupled to the microscope was used. For SEM samples were embedded as follows: A fresh sample was fixed with 2.5% glutaraldehyde. Subsequently samples were dried with critical-point dryer (CPD7501). The samples were then sputtered with gold (Sputter SCD 0050 (BAL-TEL)) and are ready for examination with a SEM (Leo 1530) on the electron microscope at TUHH.
For the examination with a TEM activated sludge samples were embedded according to Spurr [9]. The samples were placed in gelatin capsules. The polymerization was carried out for 24 hours at 70°C. Ultrathin cuts were prepared with an ultramicrotome (OmU2, Reichert-Jung) and a diamond knife (Diatom). The 70 - 80 nm thin cuts were placed on the copper grids with Movital (polyvinyl formaldehyde in 0.25% chloroform). Post contrasting followed for 10 minutes with 5% uranyl acetate and lead citrate in methanol. Thereafter, the samples were investigated with TEM (LEO 906e). The preparation of ultrathin cuts and the imaging were performed at the department of electron microscopy at the University of Hamburg.

IMPACT OF ULTRASOUND ON THE ACTIVATED SLUDGE AND *M. PARVICELLA AND P. AERUGINOSA BACTERIA*

The SEM analysis of an activated sludge sample from Seevetal WWTP (TS = 4.2 g/L) shows irregular flocs with plenty filamentous organisms, which lie within as well as peaking out of the flocs (Figure 6). Only at magnification of 20,000 we discover rod like and coccoid bacteria, which are embedded strongly into the matrix of extracellular polymeric substances (EPS). We can also see that the impact caused by ultrasound increases as the energy input rises: at 10 Wh/L the floc structure is more or less dissolved and only short fragments of the filaments remain, Figure 7. At 20 Wh/L the floc structure appears completely destroyed, we can see single bacteria cells, see Figure 8. Here no filaments exist anymore.

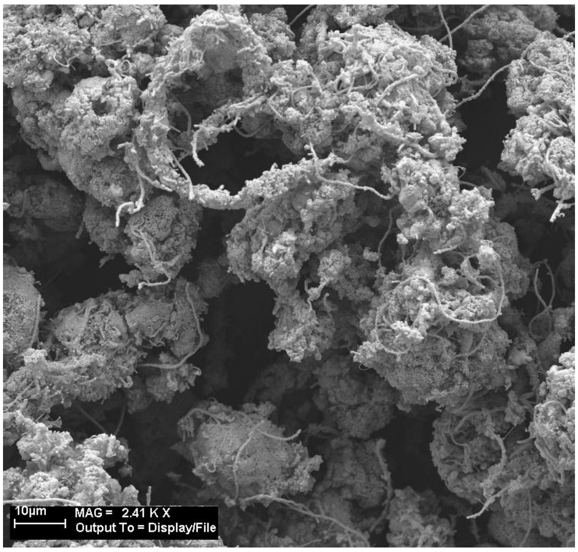

Figure 6: SEM image of the activated sludge from Seevetal WWTP (untreated).

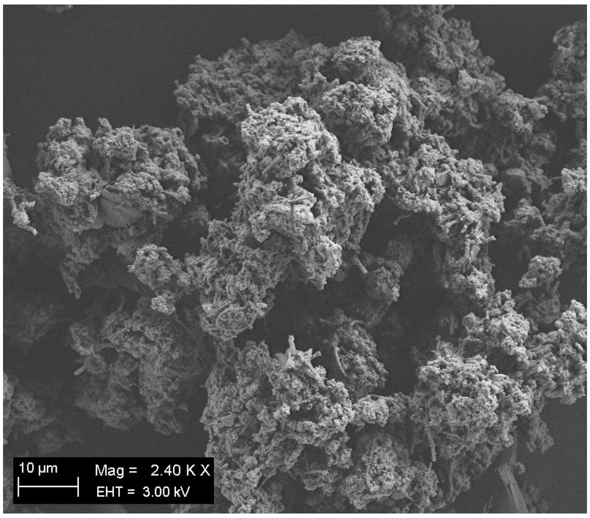

Figure 7: SEM image of the activated sludge from Seevetal WWTP (sonicated with 10 Wh/L).

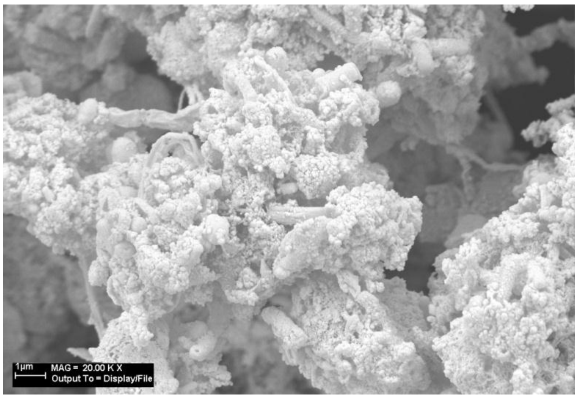

Figure 8: SEM image of the activated sludge from Seevetal WWTP (sonicated with 20 Wh/L), Magnification is 20,000.

Figure 9 depicts the release of DOC and TN into solution as function of the ultrasound energy. The graph demonstrates clearly that already at rather low energy input soluble carbon and nitrogen are generated from disintegrated cells, meaning substances from within the cell like protein (enzymes, structural protein), transport proteins bound to membranes, as well as EPS, which basically are composed by proteins.

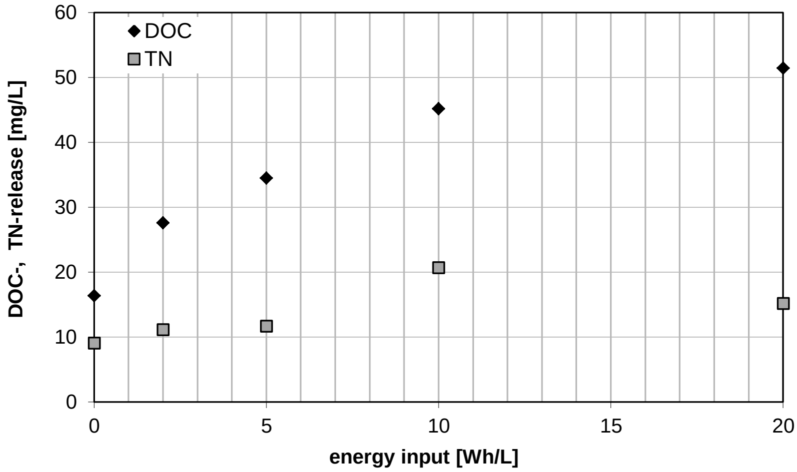

Figure 9: DOC-, TN-release of the activated sludge due to ultrasonic treatment.

The light microscope does not allow to distinguish in detail morphological elements of *M. parvicella* cells. SEM analysis does provide this information. *M. parvicella* filaments do not possess a sheath (glycocalyx). They are composed by firm compartments having a diameter of 0.5-0.7 µm. We can clearly see the septum, Figure 10. 2 Wh/L ultrasound energy applied

Feldfunktion geändert

on *M. parvicella* suspensions does not harm the cell walls, however there is an impact because the cells appear compressed. At 5 Wh/L the cell wall is being stressed and appears ragged, furthermore the cells are deformed and show bumps. Filaments now are disintegrated into short fragments. TEM analysis shows that the cells membranes are breaking often near to the septum.

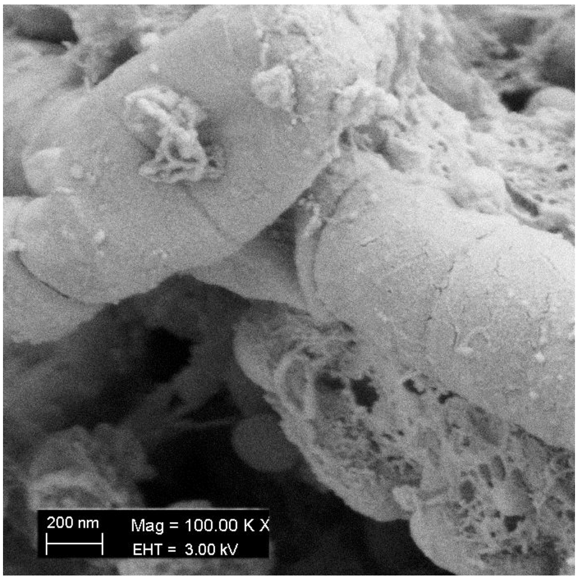

Figure 10: SEM image of *M. parvicella* (untreated).

Our SEM photos show *P. aeruginosa* cells as 1 to 2 µm long rods, partly equipped with flagella and fimbriae. In a higher magnitude the typical structure of a gram negative outer cell membrane clearly can be seen (Figure 11). At 5 Wh/L energy input we can see a number of cells with round damaged dots on the outer membrane, some cell membranes appear like pierced. Here again increasing the ultrasound energy provokes an increase in number and magnitude of damaged cells. Contrarily to *M. parvicella* TEM did not allow to conclude whether *P. aeruginosa* cytoplasma is being attacked without having destroyed the cell wall. Figure 12 is a cross (a, b) and a longitudinal cut (c, d) of a *P. aeruginosa* cell before and after sonication with 5 Wh/L and 20 Wh/L resp. We may think that, as a result of sonication, nucleoid is precipitated. The cell wall remains well.

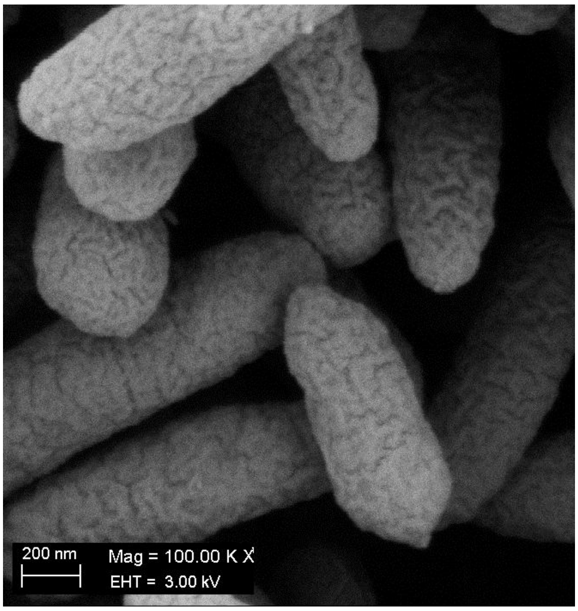

Figure 11: SEM image of P. aeruginosa (untreated).

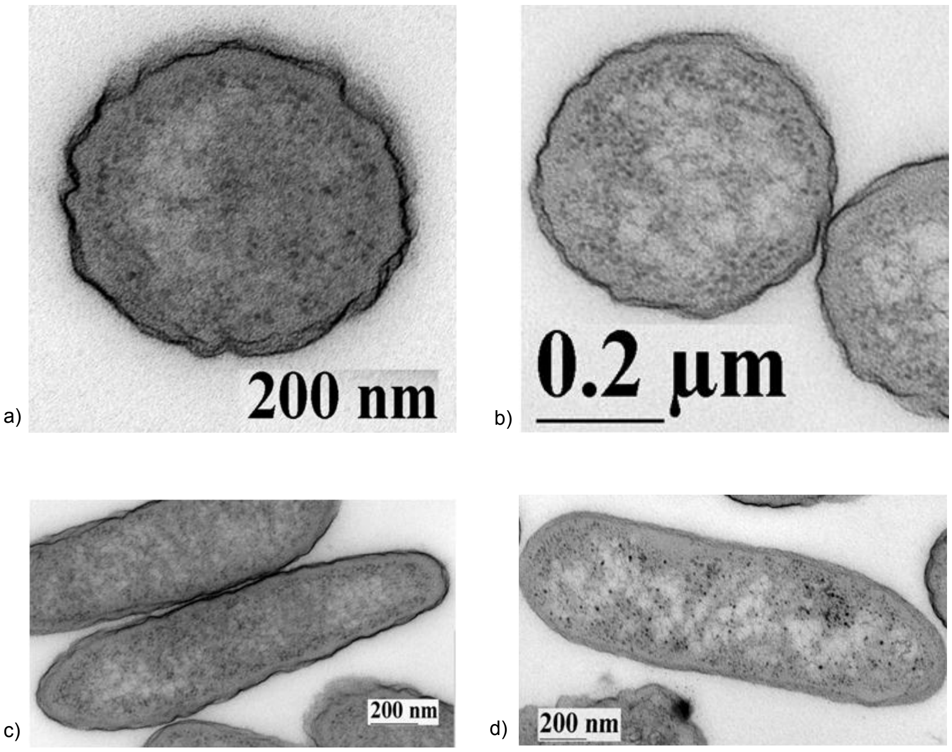

Figure 12: TEM image of P. aeruginosa cell before and after sonication with 5 Wh/L and 20 Wh/L resp., cross (a, b) and longitudinal cut (c, d).

CONCLUSION

Treating activated sludge biomass with low ultrasound energy causes de-aggregation of the sludge flocs creating favourable morphological conditions for substrate and oxygen diffusion. This leads to increased bacterial activity. However, some micro-organism species may suffer already at low energy dose (take 2 Wh/L) and cell cytoplasma can be released without obviously damaging the cell walls, for example *M. parvicella*.

At that point we do not attribute the damage of the cells to hard cavitation (implosion of bubbles creating high powerful shear forces in the liquid), since the applied ultrasound energy is low, meaning short sonication time and few cavitation events as compared to the very high number of bacteria cells in the suspension. However there is constant stress on the biomass suspension by the oscillating high power ultrasound causing wave pressure variation between +/-6.6 bar (in this particular case). We have to keep in mind that this pressure change happens 20000 times per second for minutes. The cell wall structure does not show much influence here, which is surprising if we consider that a gram positive cell wall is reported to be able to bear up to 30 bar pressure and a gram negative cell wall up to 7 bar, [10].

Eventually we have shown that low energy high power ultrasound can stimulate aerobic activated sludge biomass by creating favourable conditions for diffusion of oxygen and substrates and increasing enzymatic activity. A new finding is that the release of substances from within the bacteria cells can occur without observing damage of the cell walls. This phenomenon is due to the special characteristics of high power ultrasound which not only produces powerful destructive acoustic cavitation but first of all imposing stress on the bacterial population by considerable permanent oscillating pressure.
There might be many new developments ahead of us regarding the useful application ultrasound in environmental engineering in general and aerobic or anaerobic biological processes in particular.

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