Final Report Summary - ROUTES (Novel processing routes for effective sewage sludge management)

Executive Summary:
The Routes project was addressed to set up new technical solutions for solving typical problems of WWTPs of different capacities. To this end, 10 reference-scenarios (2 for small, 4 for medium and 4 for large plants) were compared with parallel new scenarios including new techniques and strategies under study. This comparison was carried out both from technical and environmental point of view. Quality of the sludge deriving from the investigated enhanced stabilization processes was assessed including heavy metals and organic micropollutant (emerging and usual ones) concentration, as well as phytotoxicity and ecotoxicity. Specific attention was also paid to the performance of the different enhanced stabilization processes on hygienization, including the possible re-growth of pathogens during sludge storage. New molecular tools were tested for a rapid assessment of the presence of pathogens and pathogens indicators. Risk assessment due to the sewage sludge agricultural use was carried out in relation to the fate of metals and organic micropollutants in sludge amended-soil and to possible effects on soil fauna after sludge application for several years.
Technical assessment of the 10 scenarios highlighted that there are many solutions of convenient application able to reduce sludge production, as the use of a sequencing batch biological granular reactor (SBBGR), of an anaerobic side-stream reactor in water line or of alternated cycles in water and sludge line. Removal of ammonia from liquid sludge side-streams after anaerobic digestion is convenient too. In case of primary and secondary sludge separation, which is one of the possible solution, three processes were tested for secondary sludge enhanced stabilization. Ultrasounds pre-treatment before the dual stage mesophilic + thermophilic anaerobic digestion, thermal pre-treatment before thermophilic digestion and anaerobic + aerobic sequential digestion process did not show significant differences. The potentially critical items are consumption of raw materials and reagents and thermal energy consumption for primary sludge wet oxidation, which can be recovered in case of demand by surrounding users but with some additional infrastructure complexity. The best solution might be to use directly the available heat of the liquid side-stream to increase the temperature of the biological process needed for its treatment. Hybrid disintegration (alkali + hydrodynamic) followed by dual digestion (mesophilic + thermophilic) to treat mixed sludge appeared promising and convenient as well. Environmental assessment gave different conclusions. Some new scenarios appear clearly better than the reference ones in spite of the previous technical analysis and, on the other side, those scenarios with positive technical assessment did not appear to be environmental friendly. In any case, no-one of the studied new scenarios appears negatively affected by all the impact categories (global warming potential, eutrophication potential for surface waters, marine eutrophication potential, terrestrial eutrophication potential, acidification potential and photochemical oxidant formation potential) but only by some of them. Application of any new technique therefore requires a preliminary technical assessment fixing energy, personnel, and disposal unit costs fitting the specific local situation. The specific circumstances can also address the impact category/categories more suited for the case. The practical results of ROUTES was to set up a rigorous methodology for technical analysis and LCA including all the variables influencing implementation of the technique at practical scale.

Results of phytotoxicity and ecotoxicity can take to controversial results, depending on the species under investigation and on the adopted tests. It was not possible to find a consolidated trend regarding the influence of the enhanced stabilization processes on such bioassay tests. In any case, toxicity of sludge samples to A. globiformis soil bacterium, reported as median effect concentrations (EC50), occurred at concentrations mostly higher than maximum allowed sludge application rates. For some treatment techniques, a certain toxicity reduction was observed by an increase of EC50 between untreated and treated sludge samples. This was evident for the anaerobic + aerobic combined processes, and partly for thermal hydrolysis + thermophilic digestion and hybrid disintegration + dual digestion (mesophilic + thermophilic). These bioassays should be used, therefore, with caution also with regard to the required sample quantity.

Activities on disinfection assessment of different processes pointed out that the following criteria can be used: absence of Salmonella in 50 g wet weight and E. coli lower than 500 CFU/g dm. Somatic coliphages (SOMCPH) can be used more efficaciously than enteric viruses whose enumeration in sludge sample is very difficult due to their low number. As the ratio between SOMCPH and enteric viruses is in the range of 104 - 105 it was assumed for disinfection processes the additional criterion of 104 SOHCPH.

Many activities [biopolymer production, wet oxidation, co-digestion with organic waste] produced valuable results already applied on full-scale plants (wet oxidation and co-digestion) and on pilot plants (biopolymer production). Microbial electrolytic cells (MEC) seem to have an interesting field of application for recovering products from wastewaters (methane, ammonia) reducing at the same time sludge production.
Project Context and Objectives:
The Routes project was addressed to assess new routes in wastewater and sludge treatment focused on the following main objectives:
• setting up criteria for a sustainable sludge management in different situations according to the size of the WWTP, to the presence of contaminants, to the type of wastewater treatment, to the local perception and legislation;
• optimization of the sludge quality for agricultural use by producing a clean and stabilized sludge with attention to organic micro-pollutants, hygienic aspects and other properties that can have an impact both on health and environment;
• minimization of the sludge to be disposed by innovative technical solutions based on different approaches, either on the water or sludge treatment lines;
• recovery products in sludge processing to be potentially commercialized, such as ammonium sulphate and biopolymers;
• assessment of the technical and environmental sustainability of wet oxidation;
• assessment of fate and effects of some organic and inorganic micropollutants in sludge amended soil to address the EU policy on sludge utilization in agriculture either directly or through a preliminary composting together with other organic wastes (bio-waste directive);
• establishing a set of criteria for assessing eco-toxicity and phytotoxicity and their usefulness as practical tests for assessing the sewage sludge quality;
• comparing technical and environmental aspects of ten advanced flow sheets for sewage sludge management with reference ones depending on the capacity of WWTP, quality of wastewater originating the sludge and on the type of area where is located the WWTP (sensitive or less sensitive).

Activities were organized into seven WPs. Scientific activities on sludge processing were carried out in WP1, mainly addressed to prepare sludge for agricultural use, and WP2, where a panel of different techniques were studied and set up to minimize sludge production, to recover materials during sludge processing, to set up an innovative wet oxidation process for a safe disposal of sludge, not any more recoverable. A process of co-digestion of sludge with organic waste from separate collection was also studied in WP2. This process is eligible for application when digesters of sludge lines are under-loaded, with the aim to maximize biogas production. In this case, digestate quality is considered for its potential recovery as green compost.

In WP3 different processes were studied either on pilot or full scale. Three of these processes were also included in the investigations of WP2, i.e. wet oxidation, ammonia stripping and anaerobic co-digestion. In WP3 alternated cycles (AC) on water and sludge line were investigated on the full-scale plant of Grottamare (Marche Region in Italy). AC on water line means that through a control of dissolved oxygen (DO) and oxygen reduction potential (ORP) during biological process with nitrogen removal, it is possible to save energy for aeration and to produce less biomass. AC on sludge line means that a part of the sludge recycle is fed to a reactor where the control of ORP causes a stress to the biomass thus decreasing production through the known mechanism of uncoupled biomass growth. Sludge pumping was studied with the idea that in some local conditions sludge can be treated in a centralized plant thus avoiding the full treatment on the site of production. In these cases sludge pumping can be a good solution for avoiding truck transportation and related environmental impact. Either sonication or thermal hydrolysis were studied as alternative solutions for improving sludge pumping characteristics. In this way, this pre-treatment would be useful also for increasing the digestion performance in the final treatment plant where the sludge is...
be useful also for increasing the digestion performance in the final treatment plant where the sludge is finally treated.

WP4 was addressed to study the fate of contaminants in sludge-amended soil through batch and column percolation tests. Equilibria and kinetics of sorption/desorption were assessed in different conditions. WP4 included the assessment of ecotoxicity and phytotoxicity of sludge samples mainly derived from activities of WP1. Important activities of WP4 included also the study of pathogen and pathogen indicator regrowth during sludge storage. Finally, laboratory data on fate and effects of micropollutants in sludge-amended soils were compared with data obtained from various fields sites treated with sewage sludge for several years.

WP5 was addressed to the technical, economic and environmental assessment of most of the techniques studied in WP1, WP2 and WP3. These techniques were included in ten flow sheets where a reference scenario was compared with an innovative one including the proposed advanced process. Mass (total and volatile solid, water, nitrogen, phosphorus, COD) and energy balance of each of the flow sheets (two for small size, four for medium and four for large plants) were firstly evaluated and cost comparison was performed for the assessment of technique feasibility. These activities were carried out inside the benchmarking working group, composed by four partners. The whole set of data was then provided to the partner (UniChalmers) involved in environmental assessment where different impact categories were considered (global warming potential, acidification potential, eutrophication potential and photochemical ozone creation potential). Moreover, the LCA identified a number of areas where further assessment would be needed, in particular connected to the coverage of relevant life cycle impact categories when a reference scenario where sludge is incinerated is compared with an innovative one where sludge is used in agriculture. Toxicity impacts resulting from emissions of heavy metals and organic micropollutants was one of the areas highlighted as potentially important. One of the ambitions of the ROUTES project was also to explore the inclusion of pathogen risk in LCA in the context of wastewater and sewage sludge management.

WP6 was aimed to guarantee the largest diffusion of results involving targeted groups of stakeholders especially during two dedicated conferences. The conventional diffusion tools, like papers on scientific journals, communications at specialized workshop and conferences, newsletters, factsheets, brochures, flyers, posters, were diffused in two workshops inside important conferences and preparation of stands inside two important fairs. A web site (www.eu-routes.org) was kept up-dated with three sections: one public, one dedicated to stakeholders (board of end users) and one for the partners.

Management of the project was carried out in WP7 where activities were carried out by the coordinating person and his team including one full time and one-half time scientists. The Coordinating person has continuously interfaced with the European Commission. The management team was set up to collect the documents from the beneficiaries (deliverables, contributions for the mid-term report, activity report, executive research plans, papers for the organized workshop and conference of end-users, review of the papers), and to organize the meetings and prepare all the documents for the Commission and the beneficiaries.

Project Results:
Figures and tables are provided in a separate file

WP1
Work package 1 included the following activities with the aim to maximize sludge quality and biological stability improving at the same time energy recovery:
Stability improving at the same time energy recovery:

a) Sequential anaerobic + aerobic digestion, as potential promising alternative to the conventional one-phase process;
b) Sludge decontamination by sonolysis or ozonation;
c) Enhanced stabilization processes, i.e. i) sonication pre-treatment before a dual stage mesophilic + thermophilic semi-continuous digestion, ii) thermal hydrolysis + thermophilic semi-continuous digestion, and iii) hydrodynamic cavitation pre-treatment before batch dual stage digestion (mesophilic + thermophilic).

Objective was to select the optimum operating conditions to assess the minimum solid retention time necessary to produce a stabilized, hygienic product, satisfactory gain of biogas. Effectiveness of viral indicators and pathogens removal was assessed by culture-based methods and quantitative real time PCR assays that present advantages regarding speed and specificity in comparison to cultivation-based methods. The biodiversity of microbial populations in innovative mesophilic/thermophilic temperature phased anaerobic digestion was evaluated by means of Fluorescence in Situ Hybridization (FISH).

WP1 was subdivided into seven activities.

In the activity 1.1 new molecular tools based on FISH (fluorescence in situ hybridization) were developed and evaluated by Vermicon for the fast and specific identification of indicators and pathogenic bacteria in sewage sludge samples. The developed tools contributed to assess the performance of the different applied treatment techniques of the project in terms of required hygienic aspects. The molecular approach was developed for the quantitative and fast detection of E. coli and Salmonella in sludge samples. Approach was based on micro-colony analysis with specific FISH probes, which result in a distinct specific cell count per volume or cfu/g wet weight. With these new tools, a time to result reduction of 50% for E. coli, 60% for qualitative analysis of Salmonella and 75% reduction for quantitative Salmonella approach was achieved. For the detection of Clostridium perfringens spores a new FISH-based test kit was developed, which is already commercially available. Detection limits of the developed quantitative micro-colony FISH method for salmonella in sludge samples range between 10 cfu and 5 × 10^3 cfu/g wet weight (ww). In general, the performance and reproducibility of newly developed methods was comparable to conventional cultivation methods.

Activity 1.2 was addressed to the removal of Linear Alkylbenzene Sulphonates (LAS), nonylphenol and nonylphenolethoxylates (NP/NPEOs) and brominated flame-retardants directly in sludge via oxidation processes as sonolysis or ozonolysis.

LAS were significantly oxidised by applying 200 kHz ultrasounds. Native surfactants removal varied from 31 to 42%, after the ultrasound treatment with a specific energy input of 19,500-45,000 kJ/kg TS, while no degradation occurred for non-ionic surfactants confirming their resistance to oxidation. High-frequency ultrasound pre-treatment was effective also in terms LAS removal during digestion in comparison with untreated sludge operating at a feed to inoculum ratio (F/I) of 0.3. The positive effect of sonolysis was not evident at F/I = 1.

Ozonation of sludge for degradation of brominated flame retardant pollutants produced similar results both for mixed digested and primary sludge. During sludge ozonation, most of all monitored PBDEs were effectively removed when high ozone dosage was applied. Initially most of the halogenated organics appeared to be adsorbed onto the sludge solid particles and during ozonation a fraction of the halogenated organics (up to 40% at 0.11 g ozone/g TSS for the mixed digested sludge) were transferred to the aqueous phase. The investigation about identification of degradation products did not lead to
Activities 1.3 on enhanced stabilization processes highlighted that the innovative two-stage mesophilic + thermophilic anaerobic digestion process showed better results in terms of solids removal rates and methane production with respect to conventional single-stage digestion, irrespectively on the presence of a pre-treatment by sonication. The ultrasonic pre-treatment showed a significant gain in methane production operating at high organic loading rate due to the conversion into biogas of the soluble VFAs produced in the first mesophilic stage. The only drawback of this intensive stabilization process resulted in higher soluble ammonia loads with respect to conventional treatments.

Sequential anaerobic + aerobic treatment was very effective in terms of VS removal due to their additional aerobic degradation coupled with the temperature increase of the 2nd aerobic stage. The good performance of nitrification-denitrification was confirmed also with kinetics that can make this process suitable for application.

Figure 1 summarizes the main results in terms of VS reduction and specific biogas production (SGP) of the enhanced stabilization processes performed by CNR-IRSA. It must be pointed out that the same waste activated sludge was used in these tests. Hybrid chemical (pH 9) + hydrodynamic (30 min) disintegration pre-treatment and two stage anaerobic digestion (mesophilic + thermophilic) was tested by UniBielsko in batch digestion tests (Figure 2). In order to show the effectiveness and impact of adding disintegrated sludge on the investigated dual stage digestion, parallel experiments have been carried out in each operating condition. The highest improvement (+40% of biogas production) of the digestion process was obtained by adding 30% of disintegrated sludge (WASD) to 20% of digested sludge as inoculum (DS) + 50% of raw waste activated sludge (WAS), with HRT of 7 d for the mesophilic stage and 17 d for the thermophilic one. Typical performance in terms of biogas yield production is shown in Figure 3 depending on the mesophilic HRT. The fraction of the disintegrated sludge affected the mesophilic fermentation and a slight reduction of somatic coliphages and a gradual decrease in the population count of Salmonella spp was observed. After the two stage anaerobic digestion plus pasteurization, sludge was completely hygienized.

The application of the newly developed probe CTH485 allowed monitoring progressive increase of Coprothermobacter proteolyticus during the thermophilic digestion. During thermophilic anaerobic digestion, mainly syntrophic associations between Archaea and Bacteria were established. The presence of a highly specialized microbiota resulted in a low biodiversity that can make thermophilic systems more susceptible to sudden changes and less prompt adapting to operative variations like a shift in substrate composition, as seen in the two-stage process. On the contrary, a higher biodiversity associated to mesophilic anaerobic process may determine a more rapid adaptation to HRT changes targeting the optimization of mesophilic pre-treatment.

The enumeration of E. coli of ROUTES untreated secondary sludge samples (Figure 4) clearly shows that requirement of 6 log reduction of E. coli by enhanced stabilization processes cannot be assessed. The E.coli concentration was in fact rarely above 106 CFU/g dm in the untreated sludge and therefore reduction to below detection limit (ranging between 10 and 102 CFU/g dm depending on feed concentration) can be measured.

Table 1 shows the percentage of ROUTES treated sludge samples conform to the proposed microbial indicators removal requirements or concentration limits. The sonication and thermal hydrolysis pre-treatments as well as the double mesophilic-thermophilic digestion and combined anaerobic-aerobic stabilization were evaluated in comparison with the single thermophilic (10-15 d SRTs) and mesophilic
Stabilization were evaluated in comparison with the single thermophilic (10-15 d SRTs) and mesophilic digestion (at 5 and 15 d STRs) operated respectively before thermophilic digestion and aerobic stabilization. With the exception of mesophilic digestion and sonication pre-treatment all the analysed treatments were able to remove 2 log of E. coli, in all or all but 1 sample, producing sludge considered suitable for agricultural use. As expected, among the enhanced stabilization processes thermal pre-treatment was the most effective one for achieving sludge hygienization producing sludge suitable for agriculture use with respect also to the ROUTES standard on SOMCPH (<104 PFU/g dm). Notably, mesophilic + thermophilic digestion and anaerobic + aerobic combined stabilization are effective to obtain an increased hygienization performance with respect to a single digestion step.

Table 1 shows that thermophilic digestion, as single step or in combination with mesophilic digestion, is a process complying the ROUTES standards on pathogen concentration, i.e. E. coli < 500 CFU/g dm and Salmonella absent in 50 g wet weight. However, combined mesophilic + thermophilic digestion gave better results in term of SOMCPH producing sludge conform to the proposed limit (<104 PFU/g dm) more frequently than thermophilic digestion. Similarly, the aerobic stabilization produced treated samples conform to the E. coli (<500 CFU/g dm) and SOMCPH limit more frequently than the simple mesophilic digestion.

In conclusion, regarding indicators of the stabilization processes for pathogens removal, SOMCPH was the only microbial parameter allowing a clear assessment of the increased hygienization performances obtainable by thermal pre-treatment with respect to the conventional digestion process. These results may provide effective evidence for an estimation of health risk due to sludge agricultural use.

WP2

Work package 2 was aimed at evaluating and optimizing innovative technical solutions for sludge minimization and disposal to be applied on small, medium and large WWTPS. WP2 includes evaluation and development of process techniques that lead to sludge minimization inherently within the wastewater treatment process and transformation of wastewater and sludge constituents into valuable by-products, such as, VFAs, biopolymers, biogas, and ammonium sulphate.

WP2 was divided into ten technical activities among six different beneficiaries: CNR-IRSA, UNIROMA1, AnoxKaldnes, INCA, UniBrescia, and EAWAG. The ten technical activities included:

2.1 Sludge production minimization by SBBGR (CNR-IRSA);
2.2 Optimization of integrated side-streams bioprocesses for sludge reduction in MBR (CNR-IRSA);
2.3 Sludge production minimization by microbial electrolytic cells (UNIROMA1);
2.4 Production of biopolymers from primary sludge and side streams from wet-oxidation (Bench-scale) (UNIROMA1/AnoxKaldnes);
2.5 Pilot scale production of biopolymers from primary sludge and side streams from wet oxidation (AnoxKaldnes/UNIROMA1);
2.6 Downstream processing of biopolymer-rich biomass for recovery of polymer (Pilot-scale) (AnoxKaldnes);
2.7 Anaerobic co-digestion of WAS and bio-waste (INCA);
2.8 (NH4)2SO4 recovery from ammonia (EAWAG);
2.9 Experimental set up of experiments on wet oxidation (UniBrescia);
2.10 Kinetic studies and process scale up of wet oxidation at pilot scale (UniBrescia).

Most activities (except Activity 2.3) were conducted in one or two main experimental stages, from which technical data and information was used in techno-economic and LCA work. After the Project Review process in September 2012, concerns about economic and environmental sustainability of Activity 2.1
process in September 2012, concerns about economic and environmental sustainability of Activity 2.1 were successfully addressed, changes in activities were implemented for Activities 2.2 and 2.7 and a re-evaluation of initial assumptions or considerations was conducted in the process of Activity 2.6. Activity 2.3 was the single one in the project focused on the development of a technical solution at a fundamental level.

The overall outcomes from WP2 obtained in the period of the project included:

For small WWTPs (≤ 20,000 inhabitants):
- In Activity 2.1 the bench-scale sequencing batch biofilter granular reactor (SBBGR) of 30 L geometric volume achieved a 80% reduction of sludge production during municipal wastewater treatment over 500 d of operation, producing an acceptable level of stabilized excess sludge (VSS/TSS = 0.55).
- In Activity 2.2 a selected process of membrane bioreactor integrated with an anaerobic side stream reactor (MBR + AnSSR), compared to an MBR with conventional aerobic stabilization of the wasted excess sludge (lab scale), led to a maximum yield reduction of 20% with benefits of improved nitrification and sludge filterability.

For medium WWTPs (20,000 < inhabitants ≤ 100,000)
- A process for ammonia recovery from sludge treatment liquids (Activity 2.8) was developed and optimized including an ammonia stripper and a CO2 pre-stripping unit by air to reduce the specific NaOH-demand in the main stripper (full scale). Although not included in the DoW, a membrane stripping (pilot scale) and absorption was also studied as alternative technique. Ammonium sulphate is a valuable fertilizer, which can be used for liquid fertilization in agriculture. Especially the CULTAN fertilization (Controlled uptake and long term ammonium fertilization) is characterized by some peculiar advantages in comparison to conventional fertilization with granulate. Therefore, the product ammonium sulphate is very in demand. Its concentration is limited to 40% salt content (higher concentration would produce crystallization of the product). In this form, the ammonium concentration is about 100 g N/L. Moreover, the fertilizer solution contains about 8% sulphur. To reach this optimal composition of the product it is of great importance to use concentrated sulphuric acid (> 60%) that is diluted by water vapour diffusing from the sludge liquid to the product solution due to the difference in the osmotic pressure.

For large WWTPs (> 100,000 inhabitants)
- Different process units (both lab and pilot scales) were developed and optimized in Activities 2.4 2.5 and 2.6 and the integrated process for the production of a biopolymers value-added polyhydroxyalkanoate (PHA) appeared to be feasible. It includes primary sludge processing by acidogenic fermentation or wet oxidation and municipal wastewater treatment by high rate SBR. A sludge reduction of 25% was achieved with sludge fermentation as preferred sludge treatment, producing a valuable biomass with PHA contents of 0.35-0.40 g PHA/g VSS.
- Wet oxidation batch tests were performed in Activities 2.9 and 2.10 on seven different types of sludge in order to study the effect of process conditions on WO efficiency. It was observed that the main parameters affecting WO process performance are temperature and reaction time (optimal values T=250°C and θ=60 min). Wet oxidation modelling led to the determination of kinetic parameters as a function of sludge characteristics (origin, VSS/TSS ratio) and a conceptual model was proposed. As far as WO liquid residue is concerned, anaerobic treatability was assessed and a COD removal efficiency of 30 ÷ 50% was obtained (HRT=33 d, pH=7÷8, CH4 content in biogas=70-75%). A residual organic content of 5-15% was still detected in the solid residue after WO. The BOD5/COD ratio of the liquid effluent after the treatment (always greater than 0.5) does not seem to be influenced by the different types of sludge.
- Many WWTPs have spare volume of sludge digesters that can be used properly for co-digestion with different organic wastes (wine lees/vinasses, food waste). Such possibility was tested in Activity 2.7 both
Different organic wastes (wine lees/vinasses, food waste). Such possibility was tested in Activity 2.7 both in mesophilic and thermophilic conditions. Results evidenced that the process is particularly stable at an OLR of 2.3 kg COD/(m³ × d) (70% lees on a COD basis) and the biogas yields could reach values of 0.59 and 0.68 m³/kg COD fed in mesophilic and thermophilic conditions respectively. The best COD removal, biogas yield and consequent stabilization of bio-waste were obtained at 55°C. Agro-waste treatment, such as wine lees, as co-substrate in anaerobic digestion with WAS allowed to improve the energy balance of WWTPs giving the chance for a proper recovery of this bio-waste. The anaerobic process showed a strong impact on the degradation of polyphenols, with higher degradation efficiency at mesophilic temperature than in thermophilic conditions. Consequently, effluent from thermophilic processes generally contains higher levels of polyphenols.

Activity 2.3 (Sludge production minimization by microbial electrolytic cells) was carried out on lab-scale and was typically considered as leading edge technique whose developing on practical scale was not included in this project. On the basis of the experimental results, a wide range of information regarding the capability of the MEC technique to treat low strength wastewater coupled with the generation of methane can be derived.

• First of all, electro methanogenesis occurred at the MEC cathode when the anode was fed with acetate and a complex organic mixture containing substrates, other than acetate, independently on the exchange membrane (proton PEM or anionic AEM) used to separate the two compartments. Generally, a higher performance was achieved when the MEC was equipped with a PEM and with acetate as the only carbon source. More in detail, the optimal operating condition between those investigated was obtained at -0.1 V vs. SHE with a COD removal efficiency of 88 ± 2% [corresponding to a removal rate of 0.95 ± 0.02 g COD/(L of anodic compartment × d)], a methane production rate of 71 ± 3 meq/d [corresponding to 0.25 ± 0.01 NL/(L of cathode compartment × d)] and an energy efficiency of 137 ± 6%. The reason of the higher performance of the PEM could be attributed to the higher ionic mobility of the proton in the electrolyte solution; the ionic mobility probably could be involved in kinetic limitation of the transport across the membrane of the ionic species to maintain the electroneutrality of the system. For this reason a more selective and high-performance membrane as the PEM could reasonable offer a lower resistance to the transport instead of a non-specific anionic exchange membrane, where hydroxide and other anions have a lower mobility in comparison to the proton.

• When a more complex substrate was used as feeding solution for the anodic compartment of the MEC, a lower performance was obtained than when feeding with acetate (in both cases by using a PEM membrane). This effect of the feeding composition is likely due to the presence of peptides and carbohydrates in the COD source that required previous hydrolytic and fermentative steps with respect to the acetate. This assumption was confirmed by the microbial characterization of anodic compartment whereby both fermentative and anodophilic microorganisms operated in strict synthrophic relationship.

• Microbial characterization of the cathodic compartment offered another interesting result, indeed only two types of archeal methanogens (Methanobrevibacter arboriphilus and Methanosarcina maze) were determined by Denaturing Gradient Gel Electrophoresis (DGGE) analysis. These results clearly indicate the possibility to preserve the methanogenic environment from chemical or microbial inhibition by using a protonic membrane to separate the anode and the cathode compartments of the MEC.

• Irrespectively from operating at optimal or sub-optimal conditions, the sludge production was always low, in the range between 0.07 and 0.17 g COD produced/g COD removed. However, the observed growth yield resulted strongly dependent on the composition of the feeding solution. Indeed, with acetate as feeding solution, the observed growth yield showed similar values (between 0.07 and 0.1 g COD
feeding solution, the observed growth yield showed similar values (between 0.07 and 0.1 g COD produced/g COD removed) at all the potentiostatic conditions investigated and with both the PEM and the AEM. Higher value (0.14 and 0.17 g COD/g COD, respectively at the two potentiostatic condition explored) were observed when the MEC was fed with a complex mixture of organic substrates, that induced a highly heterogeneity on the microbial community composition. This can be reasonably explained by the presence of non anodophilic biomass that is capable to growth without the interaction with the solid state electrode on more complex substrates than acetate. Despite of that, the observed microbial growth yield remained quite low, in the typical range of anaerobic environment. Overall, the obtained results suggest that a CH4-producing MEC can be also used to refine both the liquid and gaseous effluents of a conventional AD system. This approach is particularly attractive because AD effluents primarily consist of diluted organic acids which are ideal substrates for “electro-active” bacteria and so can be further removed to avoid recirculation of AD effluent into the wastewater treatment plant. At the same time, continuously bubbling the biogas produced from AD through the MEC cathode will supply CO2 for methane formation, that could be a strategy to refine biogas by increasing its methane content and so its energetic and economic value. Hence, coupling AD and MEC in the sludge line of wastewater treatment plant can also contribute to decrease net sludge production while increasing the energy recovery.

As for hydrogen production, the proof of principle of a Desulfovibrio paquesii biocathode was demonstrated in a range of operating conditions. In the second period, the addition of small amounts (ca. 10 mg/L or 0.16 mg/cm2 of cathode surface) of electrically-conductive magnetite nanoparticles was a viable and effective strategy to enhance the bioelectrocatalytic activity. Indeed, microbial biocathodes supplemented with a suspension of nanoparticles displayed increased H2 production rates and enhanced stability compared to un-amended ones. Cyclic voltammetry confirmed that faradaic processes involved in microbial-catalyzed H2 evolution were enhanced by the addition of the nanoparticles. Although the mechanism underlying the stimulatory effect of magnetite nanoparticles remains, at least partially, unknown it is likely that magnetite particles allow increasing the electrical conductivity of the biofilm-electrode interface, thereby enhancing the extracellular electron transfer processes.

WP3

WP3, dedicated to demonstration (DEM) activities, is subdivided into the following five activities carried out at full or pilot scale:

• Activity 3.1 Full scale tests of wet oxidation of different types of sludge and assessment ot the residues (3V Greeneagle);
• Activity 3.2 Rheology analysis and optimization of sludge pumping (Mediterranea Acque);
• Activity 3.3 Production of (NH4)2SO4 from ammonia stripping (full-scale plants experiments) (Atemis and EAWAG);
• Activity 3.4 Full scale testing of sludge minimisation by biological alternate cycles (INCA);
• Activity 3.5 Anaerobic co-digestion of WAS and bio-waste (full scale) (INCA).

All the activities produced valuable results.

Activity 3.1 was carried out on four different types of sludge (primary, secondary or mixed sludge either of municipal, industrial or mixed municipal and industrial origin) using the WO DUAL TOP® plant in Grassobbio (Bergamo, Italy) operated by 3V Greeneagle. These tests were performed to study the influence of process parameters and sludge characteristics on the WO removal efficiencies and to assess the characteristics of the main output streams. Results on the WO tests show:

• A good performance of the industrial-scale process under the operating conditions investigated, both in terms of VSS (up to 87%) and of COD removal (up to 71%).
terms of VSS (up to 97%) and of COD removal (up to 71%).

- Although the range of variability of the operating parameters is narrow at industrial scale due to industrial process requirements, pilot tests evidenced a general influence of WO efficiencies on reaction time, temperature and oxygen dosage for all kinds of sludge.
- The effluent from WO showed a good biodegradability.
- Influent COD concentration and VSS/TSS ratio influenced the COD and VSS removal efficiencies, respectively.

These results obtained at the industrial scale are coherent with those obtained at the pilot scale by Brescia University (Activity 2.9) and with the information available in literature.

The output from a WO process are a liquid effluent, a solid residue and a gaseous emission. The liquid effluent is sent to the biological plant in the same location receiving other industrial wastewater where the organic load of the two WO plants (that one where the ROUTES tests were carried out and the second one dedicated to the high organic load wastewater treatment) can be accounted in about 2/3 of the total influent organic load. This effluent is highly biodegradable considering the high aerobic removal efficiencies of the neighbouring biological WWTP, in terms of COD, BOD and TKN. This good result is also dependent on the high temperature of the biological process (25°C), due to the released heat from the quite hot liquid effluent.

The solid residue from WO is currently mechanically dewatered and disposed as a non-hazardous waste (Code 19 08 14). An experimental activity was conducted by Brescia University to evaluate the effectiveness of a washing procedure on the quality of the dried residue. Results showed a metal concentration increase of solid residue with the sludge industrial component, which on the contrary did not significantly affect metal leachability. 3V Greeneagle performed an experimental campaign to evaluate characteristics of this solid residue after its drying. The name dried “TOP filler®” was given by the producer to this solid residue considered eligible for being managed as end-of-waste material due to its hydrophobicity giving the filler a high mechanical resistance, that makes it suitable for being recovered as structural fill. This material was subjected to environmental and mechanical compliance tests to assess the feasibility of its recovery as filler for bituminous and similar aggregates, according to the Italian rule UNI EN 13043 “Aggregates for bituminous mixtures and surface treatments for roads, airfields and other trafficked areas”. The leaching tests on the samples simulating the whole life of a bituminous mixture incorporating the TOP filler® indicate no variations with respect to the conventional filler. The CE mark for bituminous materials can therefore be applied to the TOP filler®.

The vapours from WO are cooled, washed and incondensable gases are used as secondary air into the heater. The gaseous emissions of the industrial WO process derive therefore only from the heater. Data monitoring pointed out the compliance of national standards with great margin.

Finally, the energy balance of the DUAL TOP® plant showed that the energy input of the process is for 50% sludge chemical energy and for 50% energy to be supplied as electric energy (5%) and thermal energy (45%). 90% of this energy (i.e. about 270-360 kWh/m3) can be recovered from the output streams (especially from the hot liquid stream) being only 10% the energy losses.

Activity 3.2 on sludge pumping produced the following results.

The sludge samples exhibited a shear-thinning (pseudoplastic) fluid behaviour, where the apparent viscosity decreased rapidly as the shear rate was increased, and became constant at higher shear rate reaching the limiting viscosity at the infinite shear rate. The consequence is that, as the pumping intensity increases, the shear rate increases and the viscous resistance drops.

At constant shear rate, the apparent viscosity decreased for primary and mixed sludge after sonication but
At constant shear rate, the apparent viscosity decreased for primary and mixed sludge after sonication but not for the secondary sludge, where the apparent viscosity increased. This behaviour appears not to be congruent with literature.

Rheological properties of raw and sonicated sludge were also investigated at different TS concentration. Viscosity showed an exponential dependence on TS concentration both for raw and sonicated sludge. Mathematical models (Irvine, Dodge-Metzner/De Cindio-Brancato, Herschel-Bulkley) were used to interpolate head losses recorded during the pilot pumping tests and lab rheological data. The best one matching the real behaviour of the pumped sludge was the Irvine model.

TS concentration minimizing the specific pumping energy (kWh/kg TS) for untreated sludge was evaluated to be about 2.6 and 4% for primary and secondary sludge, respectively. Power saving in pumping sonicated sludge does not balance sonication power consumption for each type of sludge.

For thermal hydrolysed sludge viscosity is similar to that of a sonicated sludge with a TS content 3 to 5 times lower. Specific pumping power (kWh/m3) shows a minimum at a certain flow rate comparable to that of sonicated sludge at a solid concentration approximately 3 times lower. Assessment of convenience of a thermal hydrolysis process should be based on total energy balance, including energy for thermal hydrolysis, increased biogas production, lower energy requirement for dewatering and sludge transportation to final recovery/disposal sites). In a conventional process, energy for a complete hygienization of digested sludge should be also considered. Energy saving for pumping is only a secondary component for thermal hydrolysed sludge.

Activity 3.3 on ammonium sulphate recovery from liquid side-stream produced after sludge digestion was conducted by ATEMIS in cooperation with EAWAG (see also Activity 2.8). Central objective of the work planned was the development and optimization of a process for ammonia recovery from liquid side-streams produced after sludge anaerobic digestion by air stripping and acidic scrubbing (see principle flow scheme in Figure 4), in order to produce nitrogen fertilizer. In the first year of operation, the conventional free ammonia stripping as well as the CO2-pre stripping was optimized at the WWTP Kloten/Opfikon by Eawag and Atemis. In a first step the free ammonia stripping column was optimized in terms of temperature, pH, air demand (air/sludge liquid flow). Proportional to an increased temperature of the sludge liquid, the free ammonia elimination efficiency clearly increases as well. The relative elimination efficiency gains 5% with a 5°C increase of the sludge water temperature at a constant air to water flow ratio of 685. At a pH of 9.3 and a temperature of 60°C elimination efficiencies in the range of 90% can be achieved.

Due to the material properties of the structured packing, the columns can be operated a maximal temperature of up to 65°C. For safety reasons, a slightly lower temperature of 60°C was considered as optimal. The ratio of air to sludge liquid is an important operational parameter and has significant impact on the overall energy consumption of the plant. A higher air flow combined with a higher pH resulted in a higher elimination efficiency. With a supernatant flow of 5.25 m³/h and an air flow of about 3,600 Nm³/h at 60°C and a pH of 9.3 an elimination efficiency of about 90% was reached. This corresponds with a ratio of 685 between air (Nm³) and liquid (m³). In addition, the operation at a higher pH resulted in a higher efficiency. With the scope of energy efficient and cost effective nutrient removal at a WWTP in mind, an elimination efficiency of approx. 90% for the air stripping plant is completely sufficient. A disproportionate effort would be necessary for the elimination of the last 10% of nitrogen. This residual load can be more effectively removed in the biological section of the WWTP.

In a second step, the CO2 stripper was put in operation. Different trials with different air flows were conducted. The air flow was in the range of 50 to 200 Nm³/h. Operational results showed a significant
conducted. The air flow was in the range of 50 to 200 Nm3/h. Operational results showed a significant decrease of base demand in the range of 50% to reach the desired pH-value of 9.3. Air flows higher than 50 Nm3/h for the CO2 stripper did not result in a more efficient CO2 stripping and a further decrease of the base demand. Due to these results obtained from the trials, it was decided to operate the column at 50 Nm3/h, which corresponds to an air to water ratio of about 10 (Nm3/m3), in order to save 50% of the base demand. An increase of the energy demand was observed in the range of 15%. Free ammonia losses by the off-gas of the CO2 stripper were in the range of 2 - 3% of the total ammonia load to the plant. The results of this study were used by Atemis in the planning of a second sludge-liquid treatment plant in Altenrhein (Switzerland). A detailed comparison of the expected treatment costs for a plant with and without a CO2-stripper showed a small advantage for the plant equipped with a CO2-stripper. However, the NaOH-consumption was nearly halved, resulting in considerably lower total energy consumption by the sum of the plant operation plus the production of the NaOH. Considering the expected energy costs increase, it is likely that the CO2-stripper can be more advantageous in the future.

In summer 2011, the co-treatment of source separated urine was tested at the facilities of WWTP Kloten/Opfikon. The urine was collected at the Eawag office building in a tank in the basement. A large part of the phosphate was separated from the urine by struvite precipitation and filtration before the urine was added to the sludge liquid. It is expected that urine segregation from the rest of wastewater has the advantage of a reduced sludge production, both from phosphorus removal and from the nitrification/denitrification. The experiments showed that it is possible to treat sludge liquid with treated urine added (10% in volume), resulting in a 40% ammonia concentration increase in the liquid side-stream. However the ammonia removal efficiency decreased. That difference could be due to the urine addition itself (the urine may contain some surface active substances, which could change the mass transfer between the water and air phases) or due to the fast changes in the operational conditions. Probability an increase in the NaOH dose and a better pH control after the NaOH addition would be needed.

To get a first insight into the technique of free ammonia membrane stripping in winter 2011/12 single trials were conducted at WWTP Neugut (Switzerland) with a pilot membrane contactor by the German company Kunst GmbH. The pilot plant consists of three membrane modules in series with a total surface area of 120 m2. In these tests, two acid cycles were operated to evaluate the efficiency of two different membrane types (Figure 5). Different sludge side-streams were tested (nitrogen content in the range of 700 - 3,400 mg NH4-N/L) at different hydraulic loads in the range 2.5 - 12 L/(m2 × h). In these trials, the removal of free ammonia was very successful. The removal efficiency varied in the range 80 - 99%. Performances decrease at lower temperature and pH values. At pH 10.2 NH3 removal efficiency was almost 100%. At these high pH values, nearly 100% of the ammonium is deprotonated to free ammonia, but this entails high base consumption. All trials were conducted with a specific flow rate of 5 - 10 L/(m2 × h). Figure 6 shows the removal efficiency of the membrane-stripping unit in a long-term test (7.5 h). A high removal efficiency was observed at high pH values (average influent concentration of 840 mg N/L, pH of 10 and 10.5 temperature in the range of 54 to 56°C, flow rate 5 L/(m2 × h). The removal efficiency of the plant is nearly stable during the tests. Controlling the pH at 10.5 instead of 10 the removal efficiency increased from about 95 to 99%.

In autumn 2013 a half scale membrane contactor plant of the company Sustec GmbH was tested at pilot scale by the Neugut WWTP. The membrane plant consists of two membrane module in series (each module has about 51 m2 surface area). In addition to ammonia separation by membrane stripping, recovery of phosphorus as magnesium ammonium phosphate before ammonia stripping was
Recovery of phosphorous as magnesium ammonium phosphate before ammonia stripping was investigated. In this case CO2 stripping to increase pH and reduce base consumption is carried out by struvite precipitation. Tests with two membrane modules in series and a dosage of base in-between the modules evidenced the same removal efficiency in the two modules at the same inlet pH. The results of the trials with two pilot-scale membrane plants showed a high potential of the gas permeable membrane technique.

Activity 3.4 (INCA) was addressed to sludge production minimization by the alternated cycles process (ACSL) applied to both water and sludge lines.

The alternate cycles process applied in the sludge line (ACSL) is based on the alternation of oxic and anoxic phases to minimize, by biological way, the waste sludge production (Figure 7). The ACSL process varies the ORP value in a defined range to obtain the result. The ACSL is an evolution of the alternate cycle (AC) process, used in the water line, which target is the biological nitrogen removal by the automatically control with the alternation of oxic/anoxic phases into the same stirred reactor. The ACSL is realized in a reactor equipped with a DO and an ORP probes, oxygen supplies system and mixing devices. In this reactor, the oxygen supply is alternated with the anoxic phases: the ORP value increases until the aeration is stopped, than it decreases. The couple of these two phases constitutes one cycle. It is possible to impose how long time, as percentage of the ORP, the cycle has to be kept in each range by an automatic, also remote, control system. Through the elaboration of the ORP profile, it was calculated how long time the biomass is in each range and then modifies the time length of the aeration phase or of the anoxic one of the following cycle.

This strategy was implemented in four WWTPs upgraded to ACSL reconverting all of part of the aerobic stabilization units to treatment reactor installing submerged mixers and DO, ORP and TSS probes. The same control strategy was performed in the main water line dedicated to nitrogen removal (alternated cycles in water line i.e. AC). Heterotrophic growth yield $Y_h$ (kg VSS/kg COD) was determined on sludge grab samples from the ACSL reactor and the biological basin using a specific automatic respirometric system specifically designed and realized for the project. The $Y_h$ values were compared with the $Y_{obs}$ calculated from the solid mass balance.

The results of the tests carried out in four wastewater treatment plants (capacities 30,000, 50,000, 27,500 and 20,000 P.E.) pointed out that $Y$ observed can be reduced in the four WWTPs by 16%, 43%, 40% and 35%, with absolute values of 0.179 0.117 0.173 and 0.222 kg VSS/kg COD removed, for the four WWTPs respectively.

In activity 3.5 INCA studied the co-digestion of separately collected bio-waste with waste activated sludge in the WWTP of Treviso not equipped with primary sedimentation serving 70,000 PE. About 100 m3/d of waste activated sludge produced in this plant at 3.5-3.9% dm (63-65% VS) are co-digested in a 2,200 m3 mesophilic anaerobic reactor with up to 20 t/d of the organic fraction of municipal solid wastes from separate collection. Before digesting the organic wastes, this fraction is pre-treated in a mechanical selection line in order to remove metals and plastics and to shred the organic material. The shredded material is then mixed with the thickened waste activated sludge and, after the removal of coarse sinking material and floating plastics, is sent to the mesophilic anaerobic digester.

During the observation period the bio-waste load actually bestowed to the WWTP was variable and inconstant with an average value around 5 t/d. However, although lower than the target value, this load determined a clear increase in terms of loading rate to the anaerobic digester as shown in Figure 8. The effect of this extra load was that average OLR passed from 0.53 (sludge) to 1.0 kg TVS/m3 × d.
The effect of this extra load was that average OLR passed from 0.53 \(\text{kg TVS/(m}^3 \times \text{d})\) (sludge) to 1.0 \(\text{kg TVS/(m}^3 \times \text{d})\) (sludge + bio-waste) while the hydraulic retention time (HRT) decreased from 37 to 32 d. As a consequence the biogas yield passed from 0.13 up to 0.54 \(\text{m}^3/\text{kg TVS feed}\) and from 0.10 to 0.50 \(\text{m}^3/(\text{m}^3 \times \text{d})\). The methane content decreased from 67 to 60% after bio-waste addition, due to the high presence of carbohydrates (fruit, vegetable, pasta and bread) in this feed. All the biological stability parameters (pH, total and partial alkalinity, VFA) remained at constant levels. The presence of dioxins-furans, PCB and heavy metals in digestate was well below the standards for sludge use in agriculture, showing basically a good quality of waste activated sludge and the possibility of using also digestate for agronomic purposes.

WP4

Work Package 4 was focused on the evaluation of any possible detrimental effects on the environment due to sewage sludge agricultural use. The main objectives of WP4 were:

- Identification and quantification of selected pollutants as well as their transformation products (TPs) in sludge and sludge-amended soils;
- Assessment of phytotoxicity (germination tests) of water extracts from the sludge produced in activities 1.2 and 1.3 of work package 1;
- Assessing the ecotoxicological potential of individual sludge-born pollutants and their TPs under laboratory conditions with single species tests;
- Elucidation and quantification of different processes affecting fate, transport and bioavailability of sludge-born pollutants and their TPs in soil by laboratory experiments;
- Comparison of laboratory data on fate and effects of micropollutants in sludge-amended soils with data obtained from various fields sites treated with sewage sludge for several years;
- Monitoring of conventional and emerging organic micropollutants of sludge samples provided by WP1;
- Determination of the re-growth of selected microbial indicators and pathogens in treated sludge during storage of the sludge.

Activity 4.1 was focused on assessing the re-growth, if any. The studies revealed that there is no re-growth of E. coli during the storage of the tested treated sludge samples. Moreover, it was demonstrated that the use of E. coli as indicator microorganism for sewage sludge should be taken cautiously, since there is the possibility of injured cells to resuscitate after a pasteurization treatment in less than 24 h. On the contrary, sulphite-reducing clostridia spores (SRC) could be regarded as a good indicator microorganism to monitor the presence of pathogens in sludge since they cannot resuscitate from an injured state and show lower inactivation kinetics than cultivable E. coli. However, the high resistance of SRC discourages their use as indicators for monitoring sludge treatments. SRC cannot be regarded as good indicators as somatic coliphages for this purposes.

Summarising, the performed studies by UB within task 4.1 of the ROUTES project allow the following conclusions:

- There is no re-growth of E. coli during the storage of sludge at 22°C or 37°C for the tested finalised sludge (thermophilic, mesophilic and compost).
- Mesophilic treated sludge decreased values of E. coli higher than thermophilic sludge or compost when stored at 22°C.
- Salmonella presents kinetics of decay similar to E. coli.
- The relation of the abundance of between Salmonella and E. coli is around 1:100.
- Finalised sludge (after pasteurization) showed recovery of injured cells of E. coli when stored at 37°C during the first hours. Later, the number of E. coli decreases even under storage at 37°C.
During the first hours. Later, the number of E. coli decreases even under storage at 37 °C.

- Comparison of enumeration of E. coli by qPCR and culture methods supports the hypothesis of a recovery of injured cells and negated the hypothesis of potential re-growth of E. coli during sludge storage.
- The use of E. coli as indicator for faecal contamination microorganism in sewage sludge should be taken cautiously, since it was demonstrated that there is a distinct possibility of injured cells to resuscitate after pasteurization treatment at 55 °C in less than 24 h, when analysed by culture dependent methods.
- Somatic coliphages and sulphite-reducing Clostridium spores can be regarded as good indicator microorganisms of sludge hygienization since they cannot resuscitate from an injured state and show lower inactivation kinetics than E. coli. However, due to the high resistance of sulphite-reducing Clostridium spores, somatic coliphages are regarded as the superior indicators.

Enumeration of E. coli by qPCR as alternative method to the enumeration of culturable E. coli is not recommended due to the high stability of DNA, which is similar to the resistance of sulphite reducing Clostridium spores.

Activities 4.8 and 4.9 were focused on organic pollutants monitoring on sludge samples produced in WP1. Sludge concentrations of organic contaminants ranged between few ng/g of some pharmaceuticals up to 100,000 ng/g of phthalates, QACs, and anionic surfactants. It has to be pointed out that all the analysed samples in this study (apart the mixed sludge of the second campaign of the AA sequential process) were derived from secondary sludge, generally less polluted with respect to primary and mixed sludge, due to their biological origin. For the conventional organic pollutants (EOX, LAS, NPEs, PCBs, PAHs, and phthalates), the concentrations in the feed exceeded the recommended thresholds set in the EU Working Document (CEC 2000) in a few cases. However, during the subsequent treatment of sludge, concentrations of conventional organic pollutants decreased and final concentrations were always below the suggested threshold values in the above document. The removal efficiencies during the enhanced stabilization processes were different between the individual classes of investigated conventional organic pollutants. For most substances classes the dual-stage mesophilic + thermophilic process integrated with ultrasound pre-treatment was beneficial compared to the anaerobic + aerobic digestion, hybrid disintegration (chemical + hydrodynamic) + mesophilic + thermophilic digestion or thermal pre-treatment before thermophilic digestion option. The concentrations of emerging organic pollutants in feed sludge were already lower compared to available literature data given the origin of sludge (secondary sludge). However, note of the tested pre-treatments (hydrodynamic disintegration, thermal hydrolysis or ultrasound) had a substantial influence on the removal efficiencies for the emerging organic pollutants. Despite the mass losses (reduction of the loss of total solids) during all the treatments, a substantial increase in pollutant concentration in the final sludge compared to the feed samples was only observed for the thermal pre-treatment/thermophilic digestion and hybrid disintegration + mesophilic + thermophilic digestion process. Therefore, the dual stage, anaerobic + aerobic or mesophilic + thermophilic digestion process can be regarded as more effective in reducing the loads of emerging organic pollutants in relation to the single stage treatment options. The highest single step removal rates were observed during the aerobic post-treatment indicating a potential to increase sludge quality by combined aerobic and anaerobic treatments.

Most of the investigated pollutants are bio-transformed to some extent during sludge anaerobic digestion, more in thermophilic than in mesophilic conditions. Nevertheless, best removal performances have been observed for the aerobic digestion as post-treatment in the anaerobic/aerobic sequential process. Further research is still needed to identify the main parameters governing the removal of these organic contaminants, particularly for surfactants from solid matrices.
Contaminants, particularly for surfactants from solid matrices.

Regarding phytotoxicity assessment (Activities 4.4 and 4.5 by UniLublin) the tests were basically run on sludge extracts. The test consisted on a germination/elongation test, in which seeds of three species recommended by OECD (1984b) were used: Lepidium sativum L. (cress), Raphanus sativus (radish) and Triticum Laestivum (wheat). Briefly, on each petri dish, 15 seeds were put and 6 mL of sludge extract were added. Tests were run for 72 h under the exclusion of light. For wheat temperature was 20°C and for cress and radish room temperature was adjusted to 25°C. All tests were run in six replicates. The number of seeds germinated and the length of the roots was evaluated.

For the thermal hydrolysis/thermophilic digestion process no difference was detected for all plants between feed and digested sludge. The inhibition of germination and root growth was above 70%. Apart from L. Sativum (cress) thermal pre-treated digested sludge caused lower inhibition than the feed.

For the sequential anaerobic/aerobic process regardless of treatment, sludge caused 100% inhibition of germination and root growth of cress and radish. Reduction of germination and root growth of wheat was lower (about 20%) for digested sludge in comparison to untreated sludge. Effects of inhibition for all the plants except cress were lower for digested sludge. For cress no difference between untreated and digested sludge was detected.

For Sonolysis the effect of treatment was dependent on plant species.

Regarding the Activity 4.3 on ecotoxicity, a selection of laboratory bioassays were performed by ECT on sludge samples produced in WP1 activities. As biological testing is not a legal requirement for sewage sludge, and hence, no test systems are officially defined, biotests were selected according the international guideline for the ecotoxicological testing of waste (CEN Guideline 14735). The guideline recommends a battery of terrestrial and aquatic tests in order to account for different exposure scenarios. Eluates were produced from the sludge samples and tested for acute toxicity toward the freshwater micro-crustacean Daphnia magna and for inhibition of growth (of freshwater green algae). Testing of eluates does not attempt to assess the risk to aquatic organisms resulting from leaching and run-off of land-applied biosolids. It is rather a method to test for the toxicity of water-soluble substances in the biosolids, which may affect aquatic as well as terrestrial organisms. Toxicity to terrestrial organisms was further assessed by testing the sludge directly for inhibition of enzyme activity in the soil bacterium Arthrobacter globiformis and for avoidance behaviour of the earthworm Eisenia Fetida. The set of applied tests (Table 2) was driven by the limited amount of sewage sludge available for testing (in average 50 g dm).

In addition to sludge samples provided by partners in WP1, a biosolids sample from Canada was tested with the two terrestrial tests, i.e. the avoidance test and the Arthrobacter globiformis test. These biosolids (anaerobically digested sewage sludge that is applied to agricultural land in Ontario, Canada) were collected from a municipal sewage treatment plant in Waterloo. They are similar to biosolids applied in the field studies performed in Canada. The objective of including this biosolids sample was to compare sludge obtained by standard sewage sludge treatment with that obtained by advanced treatment methods applied in WP1.

Testing of sludge eluates with green algae and D. magna was very difficult to perform and their interpretation was ambiguous. The preparation of eluates from sludge samples turned out to be difficult, because the sludge-water mixtures were very thick and extensive centrifugation was necessary in order to separate the liquid from the solid phase. Despite centrifugation, filtering with 0.45 µm filters as required by the guideline EN 14735 was very time consuming and ineffective. The colour of the eluates was yellow to dark brown, and was probably only in the high dilutions bright enough to allow normal growing of algae. In addition, pronounced growth of microorganisms was observed during the algae tests that likely disturbed
addition, pronounced growth of microorganisms was observed during the algae tests that likely disturbed algal growth by competition and additional shading. One measure to tackle this problem could be a second filtration with 0.2 µm (sterile filtration), which means to take the risk that sludge-associated and water-eluted contaminants are lost in this step. Another solution could be the testing of Lemna spec. as aquatic primary producer instead of algae. However, this option was hampered by the limited amount of sludge.

Another obstacle in the eluate testing was the high organic matter content. The related microbial growth with its high oxygen demand in the test solutions caused a severe lack of oxygen for the daphnids. Thus, direct toxic effects from sludge-associated and water-eluted contaminants could not be differentiated from those caused by oxygen deficiency. Aeration of test vessels was employed for some eluate concentrations, but was not sufficient to guarantee for enough oxygen. Also, it promoted the risk of daphnids being trapped at the water surface.

In contrast, the terrestrial biotests proved suitable for measuring ecotoxicological effects and for detecting differences among different sludge samples and, hence, between treatment processes. While the results of the two applied tests often agreed with each other, a contradicting response as sometimes observed does not invalidate the reliability of the tests. The two test organisms belong to very different taxonomical groups and therefore it can be expected that they exhibit specific sensitivity to contaminants reacting differently to the same sample. The A. globiformis test has the positive requisite that it needs a relatively small sample volume allowing testing with a dilution series for a reliable estimation of median effect concentrations (EC50). Ecotoxicity assessment of sludge samples using A. globiformis is therefore reliable and comparable. In contrast, the earthworm avoidance test requires a rather large volume of sludge sample and could therefore only be performed at a single dosage, which does not allow quantifying the toxicity for this organism. However, the earthworm avoidance test can give a response of a key soil organism at integrative level, allowing a more straightforward extrapolation to the field. The A. globiformis test, on the other hand, measures the inhibition of a key enzyme that may be functional redundant in the soil microbial community.

The toxicity of the samples to the soil bacterium A. globiformis reported as median effect concentrations (EC50) occurred at concentrations mostly higher than maximum allowed application rates of sludge to soil in Germany and Canada. For some treatment techniques, a certain toxicity reduction was observed by an increase of EC50 between untreated and treated sludge samples. This was particularly evident for the anaerobic + aerobic combined processes For the dual stage mesophilic/thermophilic process, controversial results have been obtained for the different batches. Sonolysis tended to increase toxicity, while ozonation had no effect at all.

In comparison to the sludge samples provided by partners in WP1, the toxicity of the Canadian biosolids was very low in both terrestrial tests. These biosolids appeared safe for agricultural application with safety factors of 1,000 and 20 for A. globiformis and earthworm avoidance tests, respectively. These results show that anaerobic digestion, as it is performed for the tested Canadian biosolids, may be effective for a safe sludge production to be used in agricultural application. It remains an open question, why the most of European sludge, even after treatment, were found to exhibit much higher toxicity to A. globiformis than the Canadian biosolids. One explanation might be that toxicity is more correlated to source than to the treatment, with “source” meaning the origin (and thereby contamination) of the waste water from which the sludge is originated and type of waste water treatment (primary and/or secondary). Seasonal and daily/weekly variations may also have an impact on sludge quality. The samples of untreated sludge from WP1 showed overall and strikingly the greatest differences in toxicity even when the sludge came presumably from the same sewage treatment plant and only differed by season (e.g., 2012 versus 2013).
presumably from the same sewage treatment plant and only differed by season (e.g. 2012 versus 2013). This provides further evidence that “source” might be a key factor driving toxicity. Based on these results, “source” can be considered as an even stronger factor than the employed advanced sludge treatment method. If a reduction of potential toxicity of sludge that shall be applied to agricultural land is warranted, improving “source” control (e.g. input of contaminants into the waste water stream) may therefore be a more promising measure than further improve the end-of-the-pipe sludge treatment method. The evidence that different sludge samples produced by enhanced stabilization treatment, and therefore well stabilized and with reduced ammonium content, still exhibited greater A. globiformis toxicity provides evidence that at least part of the toxicity measured in the tested sludge samples might be caused by sludge-associated contaminants and not due to the load of organic matter at different stability level.

In Activity 4.3 the ecotoxicological effects of sludge-associated model substances (climbazole, BDDA, ketoconazole, fluconazole) were also investigated. BDDA proved to be very toxic to aquatic plants with an EC50 of 0.335 mg/L and an EC10 of 0.128 mg/L for yield biomass of L. minor. Effective concentrations for yield frond number were similar. However, Lemna was less sensitive to BDDA than green algae for which an EC50 of 0.04 mg/L is reported (UBA fact sheet 2007. In comparison to BDDA, climbazole is about one order of magnitude more toxic to L. minor with an EC50 of 0.019 mg/L for yield biomass. The toxicity in terms of EC50 for biomass increased from ketoconazole (580.6 mg/kg) to BDDA (249.4 mg/kg) to climbazole (11.1 mg/kg) and to fluconazole (9.3 mg/kg). Hence, phytotoxicity of climbazole and fluconazole appeared to be one order of magnitude higher than that of ketoconazole. With regard to the concentrations in sewage sludge found in literature, the EC10 of the model substances are by 3 (BDDA) and 1 to 2 (ketoconazole and fluconazole) orders of magnitude higher. Still, for climbazole, the sewage sludge concentration and the EC10 for B. napus are in the same range. Nevertheless, sewage sludge is considerably diluted when applied to soil and hence acute phytotoxic effects are not to be expected.

In Activity 4.2 and in Activity 4.6 fate of heavy metals and of emerging organic pollutants in sludge amended soil was studied by URCA and BFG, respectively.

The Activity 4.2 was subdivided in two tasks, i.e. Cu, Ni, Pb, and Zn availability in an acidic soil affected by lime-stabilized sludge application and Cu, Ni and Zn speciation in a sludge-amended soil using adsorption tests and XAS investigations.

In the 1st task a soil column leaching study was conducted on an acidic soil in order to assess the impact of lime-stabilized sludge on the availability of metallic pollutants (Cu, Ni, Pb, and Zn). Column leaching experiments were conducted by injecting successively CaCl2, oxalic acid, and EDTA solutions through the soil and sludge-amended soil columns. The comparison of leaching curves showed that the transport of metals is mainly related to the dissolved organic carbon, pH, and the nature of extractants. Metals mobility in the soil and sludge-amended soils is higher with EDTA than with CaCl2 and oxalic acid extractions indicating that metals are strongly bound to solid phase components. The single application of lime-stabilized sludge at rates ranging from 15 to 30 t/ha tends to decrease mobility of heavy metals while repeated smallest dose applications (2 x 15 t/ha) increase metals leaching from soil. This result highlights the importance of monitoring the movement and concentration of metals, especially in acid and sandy soils with shallow and smaller water bodies.

In the 2nd task pH-adsorption edge experiments and synchrotron-based spectroscopy techniques were used to understand the solid phase speciation of copper, nickel and zinc in a sludge-amended soil. Comparison of metal adsorption edges on the sludge-amended soil and the soil sample showed that Cu...
Comparison of metal adsorption edges on the sludge-amended soil and the soil sample showed that Cu, Ni, Zn can be retained by both soil and sludge components such as amorphous iron phases, organic matter and clay minerals, without being able to go further in the analysis. Thus, these data were combined with results at molecular scale to obtain structural information about the surface complexes formed. μ-XRF, XANES and LCF data indicated consistent differences in metal speciation between metals. While organic matter plays a dominant role in Ni binding in the sludge-amended soil, it was of lesser importance for Cu and Zn which were predominantly bound to mineral phases (iron-bearing phase). This study suggests that even if the metals can be associated with soil components (clays minerals and organic matter), sludge application will increase metals retention in the sludge-amended soil by providing reactive organic matter and iron oxide fractions. Among the studied metals, the long-term mobility of Ni could be affected by organic matter degradation (“time bomb” hypothesis), while Cu and Zn are strongly associated with iron oxides and are not mobile (“protection” hypothesis).

In Activities 4.6 and 4.7 the fate of emerging organic contaminants was studied on different scales ranging from small batch systems, to medium-sized column experiments and up to (semi-) field size scale at the irrigation field site in Braunschweig. The main focus was laid on sorbing substances with an elevated toxicological potential which are predominantly introduced to the soils by the application of sewage sludge. As reference, more polar substances – that are introduced to soils via the irrigation of treated wastewater – with only minor tendencies to sorb on sludge and soil were investigated. The main and preliminary outcome of the different fate studies of emerging organic contaminants can be briefly summarized:

• The strongly sorbing quaternary ammonium compound benzyldimethyldidodecyl ammonium chloride can be rapidly mineralized in sludge-amended soil. However, the transformation rate strongly differs between different soil types tested and a mixture of various structural isomers as TPs is formed. The detecting of these TPs in soils and sludge is the next step to go.
• The fungicide climbazole is transformed in contact with soils to one major TP which is relatively stable. However, the transformation seems to be restricted to aerobic or nitrate reducing conditions, since a transformation under anaerobic conditions without oxygen and nitrate was not observed.
• The major proportions of climbazole and its TP were sorbed to soil and hence they were rapidly dissipated from the water phase. Especially in “acidic” soils (pH < 7), the mobility of both compounds is expected to be low due the enhanced sorption of the cationic species by ionic interactions. Even after simulating strong rain events, the predominant proportion (> 95%) of climbazole and of its TP were still sorbed to soil particles.
• The sorption affinity of many emerging organic contaminants depends strongly on pH and the water composition: increased concentrations of metal cations inhibit the sorption of positively charged substances (e.g. beta-blockers), while lower pH and higher cation concentrations can favour the sorption of neutral and negatively charged substances (e.g. sulfonamides).
• Despite the continuous introduction of emerging contaminants to soil via irrigation of treated wastewater and sewage sludge, the concentrations in percolated water and groundwater on the experimental field site were generally low. Only extremely persistent and non-sorbing substances were detected in groundwater.
• Soil provides a high potential for biotransformation of emerging organic substances that are predominantly introduced via sewage sludge. Furthermore, strongly sorbing (= sludge-born) contaminants are effectively retained in the soil.

In Activity 4.10 conducted in cooperation between AAFC and ECT, field samplings were conducted at two sites in Canada in June 2012. The two sites, one in Ottawa and one in London, had been selected based...
The two sites, one in Ottawa and one in London, had been selected based on their rather similar agricultural management, geographic, climatic and pedological features and biosolids application. In both the sites biosolids (i.e. sewage sludge meeting the Canadian requirements for sewage sludge application on agricultural land) were applied the last time 44 (Ottawa) and 104 months (London) before the sampling campaign thus providing the basis for studying medium and long term effects on soil fauna respectively. After sampling, soil organisms were extracted from the soil and determined to family or species level at ECT. This work started in the first reporting period and continued in the second period. The collected earthworms were analysed for three organic contaminants (triclocarban, triclosan, and methyltriclosan - the main transformation product of triclosan). These substances were detected in considerable concentrations both in soil and in earthworms at the biosolids-treated field section, providing evidence for the persistence and bioaccumulation potential of these substances.

Selected soil fauna groups were nematodes and oligochaetes (specifically earthworms), which were analysed based on abundance and community structure. In addition, the feeding activity of soil fauna was measured as a functional endpoint by the bait lamina method.

Regarding the Ottawa site, there was evidence of a past enrichment effect caused by previous sludge amendments. This conclusion was mainly based on earthworm abundance and soil fauna feeding activity, but also on soil nematode community, reduced diversity and disturbed food web structure. Regarding the London field site it becomes apparent that enrichment effects had further ceased. However, evidence for potential negative impacts due to biosolids application grew, which may indicate that such effects were earlier largely hidden by the enrichment effects or simply needed longer to develop. This evidence was based on lower diversity within soil fauna groups, a far less structured food web, increased dominance of plant-parasitic nematodes, and the absence of nematode taxa known to be sensitive toward contamination at the biosolids site. It is currently unclear if and to which degree these findings may rather related to differences in soil texture, organic matter content or other abiotic soil parameters between the control and the biosolids part and to which degree indeed to long-term toxicity of biosolids-associated contaminants.

On the contrary, laboratory studies with Canadian biosolids had indicated that no negative effects on soil fauna would have to be expected after biosolids application with a safety margin of about 1,000. The field studies have left some doubts whether subtle changes in community structure, can really be recovered back to control level in a long-term perspective. The laboratory studies with model substances pointed out that sludge-associated chemicals can be toxic to soil flora and that the toxicity of these substances can hardly be reduced by simultaneous addition of organic material.

The studies revealed no clear indication that applying sewage sludge onto arable land affects the phytotoxicity. Although immediately after the application minor negative effects on the root growth and germination of L. sativum and S. alba seed were observed on a sandy and on a loamy soil, these effects vanished several month after the application of sludge. This approach based on a battery of bio tests for the estimation of soils toxicity after long term sewage sludge application seems not to be already used in literature. The results show that sewage sludge soil application might present a significant soil toxic effect which, depending on the test organism and soil type, may assume various values. Differences between the soils and among the test organisms can vary within very broad ranges. The results show that B. calyciflorus was the organism with the highest sensitivity to the presence of the sewage sludge. Interestingly, the toxicity of the sludge amended soils increased over time both in the case of B. calyciflorus and of some of the variants with other test species. The most popular eco-toxic test based on bacteria V. fischeri proved to be one of the least sensitive. A low level of sensitivity was found also for MARA test which, in a majority of cases, indicated a positive effect of sewage sludge, as well as for T. thermophila. As
which, in a majority of cases, indicated a positive effect of sewage sludge, as well as for T. thermophila. As bacteria and protozoans play a very important role in self-purification of waters contaminants affecting those organisms may have an indirect effect on pollutants removal in waters and soils. It should be emphasized that estimation of sewage sludge toxicity should be based on a comprehensive approach to the problem, taking into account not only various groups of test organisms but also soil type and time elapsed since the last application of sewage sludge.

WP5

In Work Package 5, the ROUTES work plan included an integrated sustainability assessment of the studied solutions: this evaluation was based on techno-economic issues and environmental aspects (Life Cycle Assessment; LCA).

During the first half of the project, efforts were put into conceptual design, data gathering and data generation, involving communication and collaboration among many different partners and a first round of technical-economic-environmental assessment. The results and the experiences from this work were used to decide on possible measures to correct the gap of some proposed techniques and on a modified assessment procedure.

A specific Benchmarking Working Group (BWG) was put together in order to handle the techno-economic assessment. The work was conducted in strict cooperation among all the “technological” partners (the University of Brescia being the Group Leader). During the first year of activity, a first benchmarking was carried out, based on at the time available data. This was reported in Deliverable D5.1 The results of this first application of the techno economic assessment were later revised, based on new experimental data and findings that arose from the research activities.

LCA activities have been performed by Chalmers University of Technology (Chalmers) in close cooperation with the BWG and other beneficiaries regarding data collection. A first environmental assessment of the studied techniques, in comparison to conventional ones, was reported in Deliverable D5.2. Based on the first results and other development in the project, LCA activities were also done in two stages.

During the second half of the project, the second benchmarking was carried out, based on the results and experiences of the first one. The methodology had been modified and the case studies had also been modified to reflect new technical developments in the project. The second LCA of the studied techniques, in comparison to conventional ones, was performed in the second half of the project. To complement the traditional LCA results, the complete inventory matrix of all collected environmental data, along with economic data for all studies systems were reported in D5.4. Finally, Deliverable D5.5 shows a new LCA approach based on toxicity, ecotoxicity and pathogen impacts along with life cycle costing.

The results from the techno-economic evaluation (reported in D5.3 Part A) and the environmental assessment (reported in D5.3 Part B) were integrated for a holistic description of the studied upgrades in D5.3 Part C.

An important part of the integrated assessment work was to outline a procedure for working with technical, economic and environmental assessment in a project such as ROUTES, in which all studied systems are model plants and some techniques are in a very early development phase. In such situations, it is often difficult and time consuming to develop an appropriate design of the WWTP and the sludge management, to describe also the surrounding system, and to find data for all the processes involved. However, since the main idea of this technical-economic-environmental assessment has been to provide guidance to the
the main idea of this technical-economic-environmental assessment has been to provide guidance to the coming activities in the project and to future work in the EU, this work is essential; and methods must be found or developed.  
The terminology shifted slightly over time and it is worth noting that terminology technical benchmarking, initially used, was replaced by technical and economic or techno-economic assessment, since it better reflects what is actually done in the project and the difficulties involved in comparing the existing to the new techniques that can only be conceptually designed. Furthermore, terminology conventional and innovative scenarios in a case study was progressively replaced by reference and new or upgraded scenarios, which also better represents what they correspond to.  
The integrated technical, economic and environmental assessment methodology developed in ROUTES was successfully applied in ten case studies. Each of these cases evaluated the consequences of upgrading a model reference WWTP simulating problems commonly experienced in actual treatment plants. In almost all the case studies, the proposed upgrade turned out to have a positive outcome for many of the assessed aspects. Some important trade-offs were identified, e.g. when new techniques are introduced that will decrease the environmental burden, this may come at the cost of additional efforts for the operator and with large uncertainties in the economic outcome. Solutions that are more complex typically experience a broader range of possible performance, thus a larger risk but also a larger potential gain.  
The methodology allows for highlighting some critical points that need further attention when similar upgrading is considered in real cases (Table 3). In many of the case studies, results were seen to be highly dependent on specific local conditions. When applied for assessing real cases, the uncertainties found in applying the developed methodology to model scenarios can be reduced by the use of case-specific data. The proposed methodology was deemed to provide valuable support in defining priorities for future wastewater and sludge management, as it is suitable for the evaluation of complex systems like the ones considered in ROUTES.  

In summary, in any future attempt to use the developed methodology, some points need to be considered in particular:  
• It is very important to assess data reliability. In particular, for the upgraded plant, the reliability of the experimental results depends on both the experiment scale (i.e. bench-, pilot- or full-scale), and the substrates characteristics (i.e. real or simulated wastewater).  
• Results are only valid for the assessed plant, which has specific characteristics of process configuration, influent quality and load, required effluent standards, and unique operative issues (e.g. overloading of anaerobic digester, poor quality of the sludge for agriculture application, high nutrient concentration in the reject water from the sludge line, etc.). The specific circumstances and constraints can be very different for different sites and can modify the role of considered parameters, leading to different final results and therefore they cannot be extended to other case studies.  
• The assessment procedure requires many calculations (biological process design, mass balance, energy balance, cost estimation etc.). A certain degree of uncertainty is therefore inherent in the procedure. This has to be taken into account when comparing the reference and the innovative solutions: slight differences are not relevant.  
• The capital cost for upgrading can vary remarkably in real situations depending on many factors and constraints.  
• Both capital and operational costs are calculated based on average loading conditions, which can be considered to correspond to actual (design) loading conditions. In case they are expected to change,
considered to correspond to actual (design) loading conditions. In case they are expected to change appreciably over the year, an extra cost has to be considered for equipment and devices oversizing and for performance loss due to working periods under suboptimal conditions.

- When sludge use in agriculture is considered, there may be impacts that are not appropriately captured by conventional environmental impact categories. Further methodology development and data collection is needed for quantitative assessments and in the meanwhile, a qualitative evaluation could be included in the environmental assessment.

The environmental assessment was performed as a life cycle assessment (LCA), as far as possible following the international standards ISO 14040:2006, ISO 14044:2006 and the International Life Cycle Data Systems (ILCD) Handbook as required in any EU projects. Studied environmental impact categories were:

- global warming potential, GWP;
- acidification potential, AP;
- eutrophication potential, EP, for freshwater, marine and terrestrial systems;
- photochemical oxidant formation potential, POFP.

In the 1st part of the project new solutions had worst performances than corresponding reference scenarios due to impacts connected to large electricity consumption. The electricity was assumed to be produced according to the average production composition of the EU-27 member states. If Swedish average electricity production had instead been assumed the differences between the reference and new solution would be much smaller.

For the new solutions addressed to sludge quality improvement for agricultural use, the reference ones present typically different sludge outlet options. Agricultural use was compared to either landfill or incineration. The environmental impact categories assessed in the first LCA were not considered to model sludge agricultural application in a comprehensive way, e.g. regarding toxicity, and further assessment were therefore planned to be attempted for the second round.

Solutions 2.1 2.2 3.1 3.2 3.3 and 3.4 presented lower environmental impact than the reference scenarios regarding GWP and POCP. Regarding eutrophication potential (EP) negative impact was seen for the solutions 2.1 and 3.1 and for acidification potential (AP) for the solutions 3.1 (with wet oxidation and biopolymer production) and 3.2 with thermal hydrolysis.

In the 2nd part of ROUTES different toxicity impacts categories were also assessed but only for two selected model systems considering that the conventional impact categories were not considered suitable to properly model some potentially important impacts related to sludge agricultural use. Furthermore, a method for inclusion of pathogen risk in LCA, based on a quantitative microbial risk assessment approach was developed and applied to the same two case studies. To complement LCA results with an economic assessment made with similar system boundaries as the LCA, an environmental life cycle costing was performed for all case studies.

For the conventional environmental impact categories, comparison of innovative scenarios with reference ones showed different situation among the case studies, but innovative scenarios never showed clearly worse result than their respective reference system. Final conclusions on many of the case studies where sludge is supposed to be used in agriculture cannot be drawn solely based on conventional impact categories. The conclusions of this second conventional LCA were the following:

- Solutions 1.1 and 2.1 display negative impact regarding eutrophication potential to freshwater due to P discharge in the effluent in comparison with the conventional scenario;
Discharge in the effluent in comparison with the conventional scenario:
• Solutions 2.3 2.4 and 3.4 (co-digestion without EBPR) display negative impact regarding terrestrial eutrophication potential due to ammonia release from field and also from WWTP (only 2.3);
• Solution 2.4 displays negative impact regarding acidification potential.

The analysis of the 10 case studies allowed drawing the conclusions shown in Table 4. The results of toxicity assessment for the two case studies 2.1 and 3.2 showed that both innovative systems perform worse using the recommended impact assessment methods, being the large difference mainly due to toxicity contribution of heavy metals (especially zinc) to agricultural soil in the innovative scenarios. It must be point out that in conventional scenarios sludge is managed by off-site incineration or co-incineration. Due to uncertainties regarding the behaviour of heavy metals in nature, e.g. regarding their bioavailability, these inorganic micropollutants are connected to larger uncertainties in LCAs than organic micropollutants. Interestingly, though, when shifting to another impact assessment method, the result for human toxicity was reversed. This difference mainly depends on the characterisation of heavy metals to agricultural fields and to air. However, due to lack of well-developed methodology and lack of data, it is still not possible to quantify the toxicity impact of the studied systems in a meaningful way.

Pathogen risk was assessed for the same two case studies (2.1 and 3.2) using the method developed in ROUTES for pathogen risk inclusion in LCA. Based on a combination of a literature-based and a model-based burden approach, the total pathogen-related burden of disease for a certain model wastewater treatment plant was estimated to be in the order of 0.2 – 9 Disability Adjusted Life Years (DALYs), i.e. number of lost years due to an illness. If 35,000 individuals (the population served by a wastewater treatment plant) would exposed to a one out of a million risk of a lethal accident (we assume an average age of 40 years and an average life expectancy of 80 years), this risk would be 1.4 DALY (i.e. 40 years lost × 0.035).

In order to see the potential importance of pathogen risk for the LCA in ROUTES, the calculated pathogen risk was scaled to the size of a medium and large WWTP and compared to the results for other impact categories for case studies 2.1 and 3.2. For case study 2.1 the endpoint impact on human health for reference scenario varied from 0.02 and 0.03 DALYS and in the innovative one from 0.35 to 0.40 where the human toxicity potential (HTP) played the major role (85-90%) in innovative scenario. For case study 3.2 the endpoint impact on human health varied from approximately 0.02 (reference scenarios) to 1.6-2.4 DALYS (innovative scenarios). Also in this case HTP played the major role in the innovative scenarios. These results were combined with endpoint characterisations of other impacts on human health, showing that pathogen risk has the potential to impact the results (in particular when sludge is land applied but also when sludge is incinerated). This implies a need for further studies to more accurately assess specific systems.

All case studies show similar or beneficial LCC results for all the innovative scenarios compared to the reference ones. The sensitivity analysis showed that the results have potential to change considerably depending on whether low or high assumptions are made (in the original assessment average parameter values was used). For example, many case studies in which electricity is sold (e.g. case study 2.1) exhibit a result that is dependent on the assumed income per kWh from sold electricity and therefore on possible subsidies.

Potential Impact:
The expected impact of the call ENV.2010.3.1.1-2 Innovative system solutions for municipal sludge treatment and management was so described: Fostering eco innovation in the European water industry.
treatment and management was so described: Fostering eco-innovation in the European water industry. Support related EU policies, strategies and plans. Reduce the overall environmental impact of wastewater treatment systems. ROUTES seems to have fully reached its objectives with significant impact according to the call.

Fostering eco-innovation in the European water industry
Regarding fostering eco-innovation in the European water industry many research activities have answered to this important goal and not only those carried out by companies. In WP2 use of SBBGR (activity 2.1) tested at scale of 30 L appears very interesting. The SBBGR application in raw domestic sewage treatment showed that it is able to perform in a single stage the entire waste water treatment train (i.e. primary and secondary treatment) carried out in the conventional plants and to assure [operating at an OLR of 1.3 kg COD/(m$^3$.x d)] removal efficiencies higher than 90% for COD, suspended solids and nitrogen. Furthermore, SBBGR reduced by up to 70% the quantity of sludge usually produced in the conventional waste water treatment plants coupled with a satisfactory stabilization level of sludge no longer requiring any further aerobic/anaerobic stabilization.

The use of microbial electrolytic cells (activity 2.3) in spite of its intrinsic characteristic of leading edge technique, appears promising for scaling-up to pilot-scale. Many aspects still need to be studied and solved considering this technique was proved with a synthetic feed simulating a domestic wastewater. The main results are related to the development of a MEC for H2/CH4 production at the cathode contemporarily using the anode compartment for substrate oxidation. Moreover, a transport of ammonium ion from the anode to the cathode to maintain the MEC electro-neutrality was also observed suggesting the possibility of ammonia removal by concentrating it at the cathode of the CH4-producing MEC.

Production of biopolymers (activities 2.4 2.5 and 2.6) was successfully proved at pilot scale in the installation of AnoxKaldnes in Brussels which can be considered the first-of-their-kind piloting prototypes for the production of PHAs as an integral part of municipal waste water treatment. The concepts of this technique can be so summarized: i) surplus biomass is harvested from the waste water treatment process rather than “wasted”, minimizing nitrogen and phosphorus recycling with liquid sludge side-streams and ii) waste water organic carbon is deviated from biomass production into value added products and renewable resources, which can be recovered or extracted from PHA-rich biomass reducing at the same time sludge production from WWTPs. The waste water treatment facility therefore becomes part of the urban/industrial water, carbon, energy and nutrient cycles rather than an end of pipe disposal system. Anaerobic co-digestion of WAS and bio-wastes (activities 2.7 and 3.5) is probably not a new approach but certainly it might be exploited to larger extent where digesters of the WWTPs have spare volume to be profitably used. Water industries should expand and include also typical processes and operation for organic waste processing especially regarding required pre-treatment needed to minimize inconveniences in sludge digesters for the presence of plastics and accumulation of inert materials. A second problem is connected with the status of the WWTP which will be converted to a waste recovery/disposal plant according to the Directive 98/2008. It is therefore of primary importance recovering digestate in agriculture. In ROUTES it was demonstrated that digestate does not present any environmental problem (presence of organic micropollutants) and therefore it might be used according to the same rules established for sludge use in agriculture.

Ammonium sulphate recovery from ammonia stripping (activities 2.8 and 3.3) carried out in close cooperation between EAWAG and Atemis, was developed on full-scale plants and many exploitable results are suitable for a prompt application by the beneficiary SME Atemis. According to the DOW the activities were addressed to optimize the operation of the full-scale plant of Kisten Opfikon equipped with
activities were addressed to optimize the operation of the full-scale plant of Kloten-Opfikon equipped with two conventional desorbing column for ammonia stripping from the sludge liquid side-streams and one column for ammonia adsorption with sulphuric acid, thus producing a solution of ammonium sulphate. Ammonia removal of 90% was considered the optimal target as the residual part could be more conveniently removed in the water line. The energy requirement of this approach compared with the energy needs in a conventional WWTP shows that the latter is more convenient. On the other hand, ammonia stripping allows recovering a fertilizer for its potential commercialization (end-of-waste criteria should be verified). A second option would be blending the liquid ammonium sulphate solution with digested sludge thus increasing its nitrogen value with benefit for agricultural use. The two mentioned beneficiaries carried out additional activities not included in the DOW using two membrane stripping pilot-scale units carried out on the WWTP of Neugut and Altenrhein. The results gained by the trials with the two pilot scale membrane plants showed a high potential of the gas permeable membrane technique for the recovery of nitrogen out of sludge liquid. The potential application will be further investigated in the programme Horizon 2020 involving also other companies (Sustec).

Activities 2.9 2.10 and 3.1 on wet oxidation (WO) were very useful in understanding the potentiality of application of this technique as disposal option instead of the more conventional one of incineration. In optimal operating conditions (typical values $T \approx 250^\circ C$, $\theta \approx 60$ min) COD and VS removal efficiencies up to 70% and 95%, respectively, were obtained. The effluent liquid appeared to be of good biodegradability as was proved by the good results of the biological section of the on-site WWTP of the Grassobbio platform operated by the beneficiary 3VGreenEagle receiving other industrial wastewater where the organic load from the two WO plants can be accounted in about 2/3 of the total influent organic load. This effluent is highly biodegradable considering the high aerobic removal efficiencies, in terms of COD, BOD and TKN. This good result is also dependent on the high temperature of the biological process, due to the released heat from the hot liquid effluent. The solid residue from WO is currently mechanically dewatered and is disposed as a non-hazardous waste (Code 190814). Recently, 3V Green Eagle performed industrial testing, outside ROUTES, aimed at evaluating the possibility to dry the solid residue from WO producing the so called dried “TOP® filler”. This material was subjected to environmental and mechanical compliance tests to assess feasibility of its recovery as end-of-waste material producing a filler for bituminous and similar aggregates, according to UNI EN 13043 “Aggregates for bituminous mixtures and surface treatments for roads, airfields and other trafficked areas”. The leaching tests on the samples simulating the whole life of the bituminous mixture with the TOP® filler indicate no variations with respect to the use of a conventional filler. The TOP® filler therefore received the CE mark for bituminous materials and similar. Finally, the energy balance example on the DUAL TOP® plant shows that the sludge chemical energy transformed into thermal energy during the oxidation process amounts to about 50% of the total energy entering the DUAL TOP® WO system. Moreover, the great majority of this DUAL TOP® total energy (about 90%, i.e. about 270-360 kWh/m3) is available for recover after oxidation.

Activity 3.2 on sludge pumping produced many interesting results which probably indirectly will be able to foster eco-innovation in the European water industry. Optimal concentration for pumping primary and mixed sludge was ascertained to be around 2.6% and for WAS around 4%. While sonication did not seem to positively influence rheology of primary and mixed sludge this was not the case for secondary sludge, in contrast with literature data. This was probably due to the release of extracellular polymeric substances (EPS) during sonication. Rheological properties of thermal hydrolysed sludge (sludge samples were provided by both CAMBI and Veolia Biothelys) were much better than those of sonicated sludge so that specific power required for pumping thermal hydrolysed sludge is comparable to that for pumping sonicated sludge with a TS content approximately 3 times lower. These results are certainly of possible
Sonicated sludge with a TS content approximately 3 times lower. These results are certainly of possible exploitation but it should be considered that pumping thermal hydrolysed sludge is of scarce interest as thermal hydrolysis process should be strictly linked to the subsequent mesophilic/thermophilic digestion and mechanical dewatering.

The concepts of sludge minimization by alternated cycles both in water and sludge line (activity 3.4) are already developed with success in many full-scale WWTPs in Italy. During experiments on four WWTPs in ROUTES it was proved that the reduction of real \( Y_{\text{obs}} \) (kg di VSS produced/kg COD removed) adopting the proposed strategy was in the range 16-43%.

Support related EU policies, strategies and plans
Activities of WP1 were strictly linked to those of WP4. In both the two WPs more fundamental activities than those of WP2 and WP3 were carried out with the scope to assess how enhanced stabilization processes might influence sludge chemical, biological, hygienic, pytotoxicological and ecotoxicological properties. From this point of view no impact in fostering eco-innovation in the European water industry can be expected but rather in supporting related EU policies, strategies and plans and in reducing the overall environmental impact of wastewater treatment systems. The crucial results obtained in this perspectives are here synthetically described.

Hygienization
The values of E.coli and the enumeration of Salmonella by MPN in the different sludge samples produced by the enhanced stabilization processes tested in ROUTES showed that thermal hydrolysis pre-treatment and all the treatments including thermophilic digestion at 55\(^{\circ}\)C easily comply with the hygienic standard established in the DOW according to the European 3rd draft of April 2000, i.e. absence of Salmonella in 50 g of final product (wet weight) and E. coli, as microbial indicator for bacterial pathogens, lower than 500 CFU/g of final product. Spiking treated sludge with Salmonella was discarded because it is not an operative and reproducible method attending to the complexity of the matrix of sludge. The same sludge samples were monitored by enumerating Clostridium perfringens as surrogated for parasites.

The efficacy of a stabilization process regarding removal of enteroviruses is difficult to assess because they are present in very low concentration even in raw sludge and therefore not detectable in the most of the treated sludge samples. On the contrary, the 100\% crude sludge samples showed instead somatic coliphages at values higher than 105 PFU/g dm. The different enhanced stabilization processes displayed somatic coliphages removal in the range 2 - 4 log10 units. The proportion between somatic coliphages and enteroviruses was confirmed to be in the range of 4-5 log units in agreement with previous studies. The comparison of measurements of both parameters allows to assess that when somatic coliphages are lower than 104 PFU/g dm enteroviruses are not present or are present at so low concentration (<0.01 PFU/g dm) that they are hardly detectable.

Quality criteria proposed by ROUTES on sludge hygienization are therefore: 104 PFU of SOMCPH/g dm, absence of Salmonella in 50 g wet weight and 500 CFU of E. Coli/g dm.

Microbial indicator evolution (E. coli, sulphite-reducing clostridia spores and somatic coliphages) and Salmonella (MPN) have been studied also during storage at 22\(^{\circ}\)C and 37\(^{\circ}\)C. It has been observed that there is no regrowth of E. coli nor at 22\(^{\circ}\)C neither at 37\(^{\circ}\)C. Moreover, the reduction of E. coli during storage at 22\(^{\circ}\)C is higher for mesophilic than for thermophilic digested sludge or compost. It was also determined that the relation between Salmonella and E.coli is around 1:104, and the decay of Salmonella is similar to that of E. coli. It was observed recovery of injured cells of E. coli during the first hours of storage at 37\(^{\circ}\)C after pasteurization, but later these values decrease significantly even maintaining sludge at 37\(^{\circ}\)C during
after pasteurization, but later these values decrease significantly even maintaining sludge at 37°C during long storage. The hypothesis of recovery of injured cells rather than regrowth was confirmed comparing enumeration of E. coli using qPCR or conventional culture methods. However, the enumeration of E. coli by qPCR as alternative method to the enumeration of culturable E. coli in storage is not recommended due to the high stability of DNA. Consequently, the use of E. coli as indicator of faecal contamination microorganism in sewage sludge should be taken cautiously, since it was also demonstrated that injured cells can resuscitate after a pasteurization treatment at 55°C in less than 24 h, when analysed by culture dependent methods.

Sulphite reducing Clostridium spores cannot be regarded as good indicator due to high resistance of their spores, especially in anaerobic environment. On the other hand, somatic coliphages showed to be good indicator microorganisms of sludge hygienization since they cannot resuscitate from an injured state and show lower inactivation kinetics than E. coli.

Ecotoxicological tests
The testing of samples provided in the first project period was continued and new sludge samples were tested in the second project period. In total, 45 sludge samples were investigated in the earthworm avoidance test, mostly at two different dosages. Full dose-response curves were produced for 58 sludge samples in the A. globiformis test. For eight sludge samples, water-based eluates were produced and tested in full-dose response curves for toxicity toward Daphnia magna and the green algae Pseudokirchneriella subcapitata.

The best reduction of toxicity towards the soil bacterium A. globiformis was achieved by the sequential process anaerobic/aerobic, followed by the dual-stage mesophilic/thermophilic with ultrasound pre-treatment and then the thermophilic digestion, alone or integrated within thermal hydrolysis. It was shown that for almost all types of treated sludge the toxicity of the final digested sludge provided a safety margin about factor 100 to the usual European application rates and hence no acute negative effective on soil organisms by sludge application to soil is expected. In addition, the potential toxicity to A. globiformis seemed to be related not only to incomplete stabilization of sludge but also to the presence of conventional (e.g. naphthalene) and emerging organic (e.g. triclocarban and carbamazepine) micropollutants. The testing of sludge eluates with green algae and D. magna was found to be very difficult to perform and not unambiguous in interpretation. The preparation of eluates from sludge samples turned out to be difficult, because the sludge-water mixtures were very thick and extensive centrifugation was necessary in order to separate the liquid from the solid phase. Despite centrifugation, filtering with 0.45 µm filters as required by the guideline EN 14735 (CEN 2005) was very time consuming and ineffective. The colour of the eluates was yellow to dark brown, and was probably only in the high dilutions bright enough to allow for normal growth of algae. In addition, pronounced growth of microorganisms was observed during the algae tests that likely disturbed algal growth by competition and additional shading. One measure to tackle this problem could be a second filtration with 0.2 µm (sterile filtration), which means to take the risk that sludge-associated and water-eluted contaminants are lost in this step. Another solution could be the testing of Lemna spec. as aquatic primary producer instead of algae. However, this option was hampered by the limited amount of sludge.

Another obstacle in the eluate testing was the high organic matter content. The related microbial growth with its high oxygen demand in the test solutions caused a severe lack of oxygen for the daphnids. Thus, direct toxic effects from sludge-associated and water-eluted contaminants could not be differentiated from those caused by oxygen deficiency. Aeration of test vessels was employed for some eluate concentrations, but was not sufficient to guarantee for enough oxygen. Also, it promoted the risk of daphnids being trapped
but was not sufficient to guarantee for enough oxygen. Also, it promoted the risk of daphnids being trapped at the water surface.

In contrast, the terrestrial bio tests proved suitable for measuring ecotoxicological effects and for detecting differences among different sludge samples and, hence, between treatment processes. While the results of the two applied tests often agreed with each other, a contradicting response as sometimes observed does not invalidate the reliability of the tests. The two test organisms belong to very different taxonomical groups and can be expected to exhibit species-specific sensitivity to contaminants and thereby react differently to the same sample. The benefit of the A. globiformis test is its requirement of a relatively small sample volume that allowed testing a dilution series and thereby often the reliable estimation of median effect concentrations (EC50). Thus, the A. globiformis toxicity of the samples is quantifiable and comparable and can be further used. In contrast, the earthworm avoidance test requires a rather large volume of sludge sample and could therefore only be performed at a single dosage, which does not allow quantifying the toxicity toward earthworms. Yet, the earthworm avoidance test measured the response of a key soil organism at an integrative organismal level, which allows a more straightforward extrapolation to the field. The A. globiformis test, on the other hand, measures the inhibition of a key enzyme that may be functional redundant in the soil microbial community.

Overall, the toxicity of the samples to the soil bacterium A. globiformis reported as median effect concentrations (EC50) occurred at concentrations mostly higher than maximum allowed application rates of sludge to soil in Germany and Canada. Yet, in several cases there was no or only a relatively small safety margin that could account for the necessary extrapolation from the laboratory to the field and from A. globiformis to toxicity expected for other (micro)organisms in the field.

For some technical treatment processes, a reduction of toxicity was indicated by the difference in the EC50 between untreated and treated sludge samples. This was particularly the case for the anaerobic + aerobic combined processes, and partly for thermal hydrolysis + thermophilic digestion and hybrid disintegration (alkali + hydrodynamic) + dual digestion (mesophilic + thermophilic). For the ultrasound pre-treatment + dual digestion (mesophilic + thermophilic), either increase or decrease of toxicity was observed in the different batches. Sonolysis tended to increase toxicity, while ozonation had no effect at all. Regarding the avoidance behaviour of earthworms, only for a few samples a negative impact on the soil habitat function was determined when added to the soil at the very high dosage of 25 g sludge/kg soil corresponding to 65 t dm/ha.

In comparison to the sludge samples provided by partners involved in WP1, the toxicity of the Canadian biosolids was much lower in both terrestrial tests. These biosolids appeared safe for agricultural application with a safety factor of 1,000 based on the A. globiformis test and of 20 for the earthworm avoidance test. This indicates that anaerobic digestion of municipal sewage sludge, the typical processing option of the Canadian sewage sludge, may be effective to produce biosolids safe for agricultural application. It remains an open question, why the most of European sludge, even after more intensive treatment, were found to exhibit much higher toxicity to A. globiformis than the Canadian biosolids. It might be argued that the sludge toxicity is more related to the source than to the processing route, with “source” meaning both the sludge type (primary, secondary, mixed) and the waste water characteristics, with possible associated pollutants, originating the sludge. In fact, the untreated sludge samples from WP1 showed overall and strikingly the greatest differences in toxicity even when the sludge came from the same WWTP and were sampled in different seasons.

Reduce the overall environmental impact of wastewater treatment systems

An important part of ROUTES (WP5) was dedicated to the technical and environmental assessment of ten...
An important part of ROUTES (WP5) was dedicated to the technical and environmental assessment of ten different treatment routes. The assessment was carried out comparing conventional/reference scenarios impacted by different sludge management problems with corresponding advanced scenarios with application of the new techniques developed in WP1, WP2 and WP3. Specific attention was also paid in the last part of the project to new impact categories including pathogens and ecotoxicity.

Sludge production is one of the main characteristics of any WWTP as environmental impact can greatly depend on sludge disposal options and of course on its quantity. One of the objective of ROUTES was in fact the minimization of sludge production which was studied in the activities 2.1 2.2 2.3 and 3.4 which were implemented as innovative solutions in the scenarios 1.1 1.2 and 2.2. The microbial electrolytic cells studied in the activity 2.3 were not integrated in any scenarios in spite their excellent performance as they were considered not yet mature for an industrial application.

The sludge production resulting from the water lines of the reference scenarios was in the range of 66-84 g/(PE × d), with the highest value for membrane bioreactor (case study 1.2) due to the suspended solids efficient removal, and the lowest values for the case studies without primary treatment (1.1 2.2 2.4 3.3 and 3.4). For innovative solutions sludge production greatly depends on the adopted wastewater treatment system: it is low for those solutions adopted for sludge minimization (solutions 1.1 and 2.2) [16 and 48 g/(PE × d), respectively] and quite high for those solutions with primary sedimentation and high rate biological treatment (solutions 3.1-B1 and 3.1-B2) needed for biopolymer production [91-93 g/(PE × d)] with discharge in less sensitive areas.

After sludge processing the production decreases to values of 45-56 g/(PE × d) (reference systems) and to values of 12-50 g/(PE × d) (innovative solutions). The most effective solutions for sludge minimization in comparison with reference systems are 1.1 with SBBGR (- 70%), 3.1 B2 with wet oxidation of primary and secondary sludge (-73%), as well as 3.2-B1 3.2-B2 and 3.2- B3 (-47-51%), all including wet oxidation of primary sludge.

It may be concluded that the new solutions for sludge minimization were proved to obtain a sludge reduction up to 70% for small plants (15,000 PE) with SBBGR and of 27% for medium plant (70,000 PE) with alternated cycles in water and sludge line. The greatest sludge reduction is obtained with thermal process like Wet Oxidation (WO) able to reduce sludge to inert solids. This solution can be applied to large plants of 500,000 PE. In these cases sludge reduction can reach values of 73% if WO is applied to both primary and secondary sludge, and to 50% if it is applied only to primary sludge.

The ecotoxicological studies performed in WP4 on sludge samples provided by partners working in WP1 allow to point out that:

a) During thermal hydrolysis and thermophilic digestion (TT process), the quality of the supernatant (in terms of ammonia and COD) was worsened due to the efficient particulate degradation. The same happens for the sludge dewaterability during the TT and hybrid disintegration + mesophilic and thermophilic digestion (UMT processes). The anaerobic + aerobic treatment (AA treatment) improved both the stability of the final sludge and the quality of the liquid fraction due to the higher removal of ammonia and COD in comparison with the other mentioned processes.

b) None of the treatments was efficient in heavy metal removal. Due to mass losses during the digestion the heavy metal concentration were slightly higher in the digested sludge compared to the feed samples still remaining much lower than the EU limits.

c) Concentration of the conventional organic pollutants (EOX, LAS, NPEs, PCBs, PAHs, and phthalates) in the feed sludge samples exceeded the recommended thresholds set in the EU Working Document (CEC 2000) in a few cases. However, during the subsequent treatment, their concentrations decreased and...
However, during the subsequent treatment, their concentrations decreased and, after the full treatment, they were always below the mentioned suggested threshold values. The removal efficiencies during the enhanced stabilization processes were different between the individual classes of investigated conventional organic pollutants. For most substances classes the dual-stage mesophilic + thermophilic process integrated with ultrasound pre-treatment (UMT) was beneficial compared to the AA, HMT or TT treatment option.

d) Concentrations of emerging organic pollutants [21 quaternary ammonia compounds (QAC), and 25 pharmaceuticals and biocides], in feed sludge were lower than available literature data. Despite total solids reduction during all the treatments, a substantial increase of the above emerging pollutant concentration after full treatment compared to the feed samples was only observed for the TT and HMT processes. AA and UMT processes can therefore be regarded as more efficient in their removal in comparison with TT and HMT treatments. The highest single step removal rates were observed during the aerobic post-treatment of the AA treatment indicating a potential to increase sludge quality by combining aerobic to anaerobic digestion.

e) The results of the ecotoxicological evaluation are mostly in good agreement with the chemical assessment. In accordance to the highest removal of emerging organic contaminants, the best reduction of toxicity towards A. globiformis was achieved by the AA process. Toxicity reduction was lower for UMT and much lower for TT. Toxicity to A. globiformis appears much more related to the conventional and emerging organic pollutant concentrations rather than to an incomplete level of stabilization.

The summarized results from the integrated technical and environmental assessment of ten treatment scenarios carried out in WP5 are here described. From the technical point of view, scenarios 1.1 (SBBGR), 2.1 (mixed sludge anaerobic + aerobic treatment), 2.2 (alternated cycles in water and sludge line), 2.3 (ammonia stripping) and 3.3 (hybrid disintegration coupled with mesophilic + thermophilic digestion) appear not to be negatively affected by specific local situation. In fact, they appear altogether less expensive than the reference scenarios, reliable and their implementation in the existing WWTPs is easy with possible modular realization, negligible additional footprint and sufficient expected lifetime. The above techniques were developed in ROUTES implementing already known processes (bio-filtration, stabilization processes either anaerobic or aerobic, ammonia stripping) with new units addressing different aspects/problems of wastewater and sludge management (sludge reduction, sludge increased stabilization, minimization of chemical consumption). All the above processes were tested with real wastewater/sewage sludge so the achieved results can considered representative of real wastewater treatment plants operation. Moreover, these techniques are non-impacted by security measures, by public acceptance and by complexity of the administrative authorization process. They require operating personnel with standard level of expertise. Consumptions of raw materials, chemicals, electric and thermal energy are not critical. Also scenarios 2.4 (sludge pumping), 3.1-B3 (acidogenic fermentation of primary sludge and production of biopolymers), 3.2-B3 (primary sludge wet oxidation, anaerobic digestion of liquid effluent, secondary sludge anaerobic+ aerobic digestion + pasteurization) and 3.4 (anaerobic co-digestion with organic waste and enhanced biological phosphorus removal in water line) can be acceptable. However, they need a more careful attention due to possible drawbacks depending on local situation and specific application. For sludge pumping (solution 2.4) the critical factors for implementing this solution at large scale are the installation of the pipeline and the CHP unit of the centralized treatment plant, requiring additional work power and authorization. Moreover, this solution might have problem of application when the sludge flow rate is different from the designed value. In fact, it might be impossible to pump a larger volume of sludge if the pipeline has not sufficient size.
In fact, it might be impossible to pump a larger volume of sludge if the pipeline has not sufficient size.

Regarding the solution 3.1-B3 with biopolymer production, the critical factors for implementing this technique are its reliability (no full-scale installations), consumption of raw materials and reagents, and higher costs under the worst conditions. The critical factors for implementing the solution 3.2-B3 (primary sludge wet oxidation and secondary sludge enhanced stabilization with sequential anaerobic + aerobic treatment) are the same as those of the solution 3.1-B3 and personnel power needed for CHP plant for biogas valorization. Solutions 3.4 with anaerobic co-digestion may be hindered by additional costs under the worst conditions and the personnel power required for energy valorization of biogas. Solutions 3.1-B1 (wet oxidation and biopolymers production), 3.1-B2 (wet oxidation), 3.2-B1 (wet oxidation of primary sludge and enhanced secondary sludge stabilization with sonication and dual anaerobic short mesophilic and thermophilic digestion) and 3.2-B2 (wet oxidation of primary sludge and enhanced secondary sludge stabilization by thermal hydrolysis and thermophilic digestion) need a deep assessment to verify convenience of their integration in an existing WWTP. In fact, many factors can restrict their use.

From the environmental point of view, the solutions with lower impacts than the reference scenarios are 2.2 (sludge minimization by alternated cycles in water and sludge line), 3.1-B1 3.1-B2 and 3.1-B3. Solution 1.2 (sludge minimization by anaerobic side-stream reactor) appears slightly worse than the corresponding reference scenarios only for eutrophication potential (EP) for freshwaters mainly cause by a marginal increase of P discharge with the effluent (+9%). Solution 1.1 appears more convenient than the reference scenario for acidification potential (AC), marine and terrestrial EP, and photochemical fog formation potential (POFP), but is worse regarding EP to fresh waters (+61% of P release with the effluent). Solution 2.3 (ammonia stripping) appears hindered by AC (+8.7%), marine EP (+15%), terrestrial EP (+36%) while is better with regard to global warming potential (GWP) and POFP. Solution 2.4 (sludge pumping) is better than the corresponding reference scenario with regard to GWP, EP to fresh waters and POFP but is worse with regard to AP, terrestrial and marine EP. Solutions 3.2 are more or less equivalent to the reference scenario as they appear less impacting considering AP and POFP, equal considering EP for freshwaters, and marginal more impacting considering GWP, only for the solution 3.2-B2 with thermal hydrolysis and thermophilic digestion (+3.5%), marine EP only for the solution 3.2-B1 with sonication and dual digestion mesophilic + thermophilic (+7.1%), and terrestrial EP only for the solution 3.2-B2 with thermal hydrolysis and thermophilic digestion (+9.1%). The 3rd solution 3.2-B3 with dual stabilization (mesophilic digestion + aerobic stabilization) is therefore the most convenient from the environmental point of view among the three proposed solution with sludge separation. Solution 3.3 with hybrid disintegration and mesophilic + thermophilic digestion appears more convenient than the reference scenario considering AP and POFP, equal considering EP to freshwaters, marginal worse considering GWP (+1.6%), marine EP (+7.1%) and terrestrial EP (+8.3%). Solutions 3.4 with anaerobic co-digestion with organic waste are more convenient than reference scenario considering GWP, AP and POFP, are equivalent considering EP for freshwaters, while are marginally more impacting considering marine and terrestrial EP (+13.9-27.8%).

No solutions show negative impacts for all the investigated impact categories. The most of them seem to be equivalent (±10%). The highest added impacts were shown by the solutions 1.1 for the EP for freshwaters (+61%), 2.3 for the terrestrial EP (+36%) and 3.4-B2 with co-digestion and enhanced biological P removal for marine EP (+21.4%) and 3.4-B1 with co-digestion without enhanced biological P removal for terrestrial EP (+27.8%).

ROUTES therefore showed the field of application of different proposed techniques and possible relevant impacts. Practical application requires a careful analysis based on local situation where many key factors can influence the final decision and the most appropriate impact scenario. It should be pointed out that the technical convenience is generally not linked to the environmental lower impact: the two different...
Technical convenience is generally not linked to the environmental lower impact. The two different approaches should be merged in a single final analysis.

For a pragmatic approach, it is possible to reach these conclusions for the techniques displaying better results than reference solutions in benchmarking and LCA:

a) The SBBGR appeared applicable for small scale plants (< 20,000 PE) but it requires a scale up to a reactor volume of at least 500 m³ as it was proved till now with maximum reactor volume of 2 m³. An application in Apulia region is now in phase of commissioning with a volume of 700 m³.

b) The solution with sequential anaerobic + aerobic stabilization can be easily implemented in medium scale WWTP up to capacities of 100,000 PE. The problem in this case is the additional volume of the aerobic reactor which can be repaid by the much better quality of sewage sludge. This solution therefore appears promising to sustain the sewage sludge use in agriculture. In this perspective, legislation can have a crucial importance to address innovative techniques.

c) ACSL in conjunction with AC in water line was already successfully applied in ROUTES on four full-scale WWTPs.

d) Ammonia stripping was also successfully applied in ROUTES on a full-scale WWTP. Energy costs seemed higher than those for ammonia removal in water line. This technique can therefore be applied on those already existing WWTPs where treatment capacity does not allow to receive additional N-NH₄ load from sludge line. Ammonia stripping is certainly a good solution to maintain the right effluent standards, and it has the additional advantage of the production of a liquid fertilizer (ammonium sulfate).

Other techniques showed a potential of application but they need attention due to possible drawbacks depending on local situations and specific applications:

e) Sludge pumping is applicable and can of course avoid transportation by truck. Sonication did not proved to reduce sludge viscosity as thermal pretreatments, which from the other side might be better allocated directly on the centralized plant. Thermal hydrolysis in decentralized WWTP (with low-medium capacities) appears now to have limited application.

f) Biopolymer production from sewage sludge was very promising and certainly is one of the best results of ROUTES. Its exploitation needs to have a careful assessment of the final step of PHA recovery from the biomass and of the resulting residues, which probably cannot be any more qualified as sewage sludge.

g) Dual step of digestion (mesophilic + thermophilic) can be applied in a new WWTP or in case of total rebuilding of digestion of an existing WWTP. In this case, the design of the two units (the first one considered as a pre-treatment) can be optimized. The advantage is to produce additional volume of biogas and a more stable sludge. This technique can be implemented in large WWTPs on secondary sludge, when the quality of primary sludge might hinder agricultural use of mixed sludge.

h) Wet oxidation currently has a lower application in comparison of conventional incineration. It has potential advantages of negligible exhaust gas production without any problem of organic micropollutants. The solid residue can also be recovered and even its state qualified as end-of-waste. The ROUTES results demonstrated that the liquid side-stream is quite heavily organic loaded (good biodegradability) and therefore has to be recycled on the top of the WWTP. Application of this technique is therefore linked to the possibility to treat the additional load.

i) Co-digestion of sewage sludge and organic waste results in a large production of biogas. In this case the WWTP is converted in a waste platform. The additional units for organic waste pre-treatment have to be included in the flow sheet. The digester needs a careful maintenance as there is the tendency to accumulate heavy material on the bottom and floating material on the top. This tendency strictly depends on the quality of the separate collection of the organic waste.
On the quality of the separate collection of the organic waste.

With regard to the other related impacts listed in the call i.e.:
- a) source control of pollutants,
- b) material reuse or disposal;
- c) public perception;
- d) innovative system solutions to be extended to the whole sewerage-wastewater treatment system;
- e) risks of sludge disposal.

ROUTES showed important results for many of them.

The source control of pollutants was not directly covered by any activities. WP4 highlighted that possible risks associated with different types of sludge (those produced in enhanced stabilization processes by WP1 and that sampled in the Canadian WWTPs of Waterloo) seem to depend mainly on the origin rather than on the type of treatment in spite of the intensity of such treatment. This confirm that the source control might be the best solution in order to produce a clean and stabilized sludge intended to be used in agriculture without detrimental effects to soils. Indeed, laboratory studies with Canadian biosolids had indicated that no negative effects on soil fauna are expected after biosolids application, with a safety margin around 1,000. The field studies have confirmed this assessment but left some doubts regarding capacity of soil fauna to recover back to control level in a long-term perspective. The laboratory studies with model substances evidenced that sludge induced toxicity to soil flora by pollutant content can hardly be reduced by simultaneous addition of organic material even at the highest rate allowed for sludge application.

Material reuse and disposal was covered by WP3. Regarding re-use, ROUTES investigated three possibilities focused on ammonium sulphate, co-digestate of sewage sludge with organic waste, and solid residue from wet oxidation. It must be pointed out that re-use is currently linked with the concepts of end-of-waste material that should comply all the four conditions of the article 6 of the Directive 2008/98 to be eligible to reach this status (see also Deliverable D6.10 specifically addressed to discuss these aspects).

Analyses of ammonium sulphate solutions produced in the activities 2.8 and 3.3 (see Deliverable D2.7) highlighted that ammonium sulfate is a valuable fertilizer for liquid fertilization in agriculture. Especially the CULTAN fertilization (Controlled uptake and long-term ammonium fertilization) is characterized by some peculiar advantages in comparison to conventional fertilization with granulate. Therefore, this product is expected to be quite high in demand. The ammonium sulfate concentration was limited to 40% salt content. Higher concentration of ammonium sulfate resulted in a crystallization of the product. In this form, the ammonium concentration is about 100 g N/L. The fertilizer solution contains about 8% sulfur. To reach this optimal composition concentrated sulfuric acid (> 60%) has to be used for membrane stripping process that is diluted by water vapor diffusing from the sludge liquid to the product solution due to the difference in the osmotic pressure. For the air stripping process, the concentration of the sulfuric acid must reach 75% to obtain the right concentration of the product ammonium sulfate. The ammonium sulfate solution produced at the Kloten/Opfikon WWTP was analyzed for micro pollutants (MPs): 10 out of 28 analyzed MPs were detected at quite low concentrations. Only 50 ppm of MPs with regard to the WWTP influent load was found in the product. The concentration of different MPs ranged from 53 ng/L (Fluconazole) to 1,633 ng/L (S-Methyl-BenzoTriazole).

Characterization of digestate produced in the activity 3.5 highlighted that PCDD/F behaviour depends on number of chlorine atoms and their position in the molecules. The most hazardous dioxin (TCDD) as well as the higher chlorinated ones are not affected by biological degradation due to their lipophilicity.
as the higher chlorinated ones are not affected by biological degradation due to their lipophilicity properties. The behaviours of PCB vary for congeners. Mass balances of some compounds closed with error lower than 25% (congeners 99, 105, 114, 156, 157, 167, 169, 170, 180, 183 and 187) but generally the amount of low chlorinated PCB increases, probably due to de-halogenation of compounds with higher chlorination degree. The differences between influent and effluent amounts are higher than 60% for Hexa-PCBs, about 70% for Penta- and Tetra-PCBs, and reaches 100% for Tri-PCBs. Heavy metals are not removed by microorganisms but conditions in anaerobic reactor can change their chemical forms. Usually they are adsorbed onto solid particles and can precipitate as sulphides and insoluble salts accumulating in the reactor depending on the metal. Cr is not retained, while mass balance for Ni and Hg indicates a retention between 25 and 50%. The others metals showed a strong accumulation on the reactor with variation between inlet-outlet higher than 50%. Final status of digestate as end-of-waste material strictly depends on the parallel status of compost with sludge. The rumours in Europe do not seem to be positive in this sense.

Solid residue from sludge wet oxidation tests was used to prepare a bituminous mixture, the so-called TOP® filler. Mechanical properties showed a very good mechanical resistance and a high resistance to water, indicating its high hydrophobicity and low metal leachability. These tests allowed to conclude that the TOP® filler does not modify the life cycle of bituminous conglomerates, neither during production, nor during transport/laying, nor during removal after use. The TOP® filler received the CE Certificate of Conformity according to UNI EN 13043 “Aggregates for bituminous mixtures and surface treatments for roads, airfields and other trafficked areas”.

Activities 2.1 2.2 2.3 2.4 2.5 and 3.4 were focused on testing innovative treatment solutions to be extended to the whole WWTP. ROUTES results confirmed that water and sludge lines are strictly interconnected as the quality and quantity of sludge depends on the presence of both macro and micro-pollutants of wastewater and on configuration of water line. Adoption of new techniques can solve some typical problems of sewage sludge management.

Dissemination activities
The following documents were prepared for dissemination, all available on the project website www.eu-routes.org:

- 5 newsletters briefly showing the main results and outputs of several project activities described for an easy comprehension to a large public without specific competences. Moreover, information on the project, on workshops and events organized by the Consortium are given as well.
- 8 factsheets with more detailed description of the most important scientific activities (benchmarking, assessment of hygienic properties, enhanced stabilization processes, recovery and fate of nutrients, fate of organic micropollutants in soil, heavy metal speciation in sludge and soil, sludge minimization, wet oxidation) and 2 factsheets specifically produced for the European Commission on the main objectives and mid-term results of the project (general aspects, mid-term achievements).
- 2 brochures (concept and the main objectives of the project, mid-term results achieved per Work Package).
- 12 posters, presented at the two International Fairs where the ROUTES Consortium set up a dedicated stand, namely Ecomondo, (November 2012, Rimini, Italy) and Pollutec Horizons 2013 (Paris, France, December 2013).
- 2 flyers (general features of the project, announcement of the 2nd End users Conference).

A Board of End-Users was set up at the beginning of the ROUTES project and has the aim to ensure the dissemination of project results and their rapid application in a more effective manner than by using the
Dissemination of project results and their rapid application in a more effective manner than by using the conventional channels of scientific communication.

At the time of the proposal submission, the Board of End Users was composed by 9 organizations. During the project time other companies have adhered to the Board and their number increased to 31 partners. The Board is constituted by companies (SMEs) involved in the design and management of wastewater treatment plants, national and international organizations, public and private research centres, professionals, and by authorities in charge for the control and issuing of authorizations for sludge management, such as regional and national environmental agencies, municipalities and ministries. The list is reported in the ROUTES web site.

Two Conferences were dedicated to the End Users. The first one was held in Rome on October 25th 2012, with an attendance of about 75 delegates and 17 oral presentations. Four of the above presentation came from the end users. The 2nd conference was held in Braunschweig (Germany) on April 2nd, 2014, with an attendance of 74 delegates and 18 oral presentations. Two of the above presentations came from the end users.

Three workshops dedicated to the ROUTES Project were organized by the Consortium. The first one was held in Florence in October 2012 during the ISWA International Solid Waste Congress where 6 oral papers were presented from ROUTES and 3 from the parallel project END-O-SLUDG. Good relationships were established between the two projects. The 2nd workshop was held as special session during the 1st International IWA Conference on Holistic Sludge Management (HSM 2013), in Västerås, Sweden, on May 6-8th 2013. 8 oral presentations were delivered by the partners of the project. The 3rd workshop was held in the framework of the Pollutec 2013 Fair in Paris, on December 3rd, 2013, where the ROUTES Consortium set up a promotion stand as well.

A training course was held in Barcelona to train all the scientists involved in the pathogens and pathogens indicator detection to use standardized methodologies in their tasks thus harmonizing the different procedures.

50 papers were published by the partners of the project (45 on peer reviewed journals and 5 on non-peer reviewed journals). Among them, 13 are going to be published on Special Issue of Environmental Science and Pollution Research, 2014.


47 participations to international Conferences/workshops with oral presentations were provided by the partners of the project.

3 thesis on specific results of project activities were discussed by students affiliated with some partners of the project.

List of Websites:

http://www.eu-routes.org

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