**Supporting information**

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**Manuscript title:**

#### Effects of engineered nanoparticles on sludge anaerobic digestion performance and associated microbial communities

**Number of pages:** 11

**Number of Figures:** 4

**Number of Table: 2**



**Figure SI-S1**. Flow schematic of the integrated activated sludge and anaerobic digestion pilot-scale plant operation with the spiked and targeted ENPs and metal salt concentration in the digestate

Transform: Log(X+1)

Resemblance: S17 Bray Curtis similarity

*Treatment*

Control

Metal salt

ENPs

*Similarity*

50

70

80

45

60

75

90

105

120

130

145

160

175

190

205

220

235

250

45

60

75

90

105

120

130

145

160

175

190

205

220

235

250

45

60

75

90

105

120

130

145

160

175

190

205

220

235

250

2D Stress: 0.11

Figure S1-S2 NMDS plot of the PLEL concentration showing temporal shift in microbial community structure in the anaerobic digesters based on Bray-Curtis dissimilarity. At 70 % similarity level, the samples were grouped into 5 discrete clusters across the three plants. The sub-clusters at 80 % similarity suggest a steady changing pattern in the biomass concentration. In addition, the tight clustering of the different samples indicates that the anaerobic and methanogenic community were less biodiverse or their taxonomic relatedness was almost similar in the three plants. The relationship between the treatments indicates a 50 % shift in microbial community at day 190 across the plants.

Table S1-S1 Summary of the most dominant OTUs of anaerobic bacterial community retrieved from anaerobic digestate

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Cluster** | **Phylogenetic affiliation** | **Order/Family/Genus** | ***Genus/Description*** | **% Match** | **Accession No.** |
| 29 | **Firmicutes** | Clostridia/Clostridiales/Eubacteriaceae/*Eubacterium* | *Eubacterium* sp E-1 | 99 | AB470313 |
| 75 |  | Clostridia/Clostridiales/Incertae Sedis XI/*Soehngenia* | *Clostridium ultunense* strain BS | 87 | NR026531 |
| 113 |  | Clostridia/Clostridiales/Peptostreptococcaceae/*Peptostreptococcus* | *Peptostreptococcus russellii* strain RT-19A | 99 | AY167957 |
| 122 |  | Clostridia/Clostridiales/Lachnospiraceae | *Lachnobacterium* sp G9 | 87 | AB730783 |
| 125 |  | Clostridia/Clostridiales/Lachnospiraceae/*Lachnobacterium* | *Lachnobacterium* sp Wal 14165 | 88 | AJ518873 |
| 139 |  | Clostridia/Clostridiales/Ruminococcaceae/*Ruminococcus* | *Ruminicoccus* sp 7L75 | 92 | AJ515914 |
| 142 |  | Clostridia/Clostridiales/Incertae Sedis XV/*Anaerobaculum* | *Anaerobaculum mobile* strain NGA | 85 | NR028903 |
| 257 |  | Clostridia/Clostridiales/Ruminococcaceae | *Clostridium orbiscindens* strain 17 | 83 | GU968170 |
| 269 |  | Bacilli/Lactobacillales/Lactobacillaceae/*Lactobacillus* | *Lactobaccillus vaginalis* strain DoxG3 | 97 | GQ422709 |
| 283 |  | Clostridia/Clostridiales/Incertae Sedis XIII/*Anaerovorax* | *Anaerovorax odorimutans* strain NorPut | 86 | AJ251215 |
| 284 |  | Clostridia/Clostridiales/Lachnospiraceae | *Anaerostipes caccae* | 87 | AB243986 |
| 294 |  | Clostridia/Clostridiales/Clostridiaceae/Clostridiaceae 1 | *Clostridium* sp L15 | 89 | AY337519 |
| 354 |  | Clostridia/Clostridiales/Incertae Sedis XV/*Anaerobaculum* | *Anaerobaculum* sp OS1 | 94 | FJ862996 |
| 358 |  | Clostridia/Clostridiales/Ruminococcaceae | *Hydrogenoanaerobacterium saccharovorans* strain SW512 | 84 | NR044425 |
| 380 |  | Clostridia/Clostridiales/Incertae Sedis XI/*Sedimentibacter* | *Sedimentibacter hongkongensis* strain K1 | 88 | AY571338 |
| 385 |  | Clostridia/Clostridiales/Incertae Sedis XV | *Aminomonas paucivorans* | 85 | AF072581 |
| 385 |  | Clostridia/Clostridiales/Incertae Sedis XV | *Cloacibacillus evryensis* gen. nov. sp. nov | 86 | CU463952 |
| 445 |  | Root/Bacteria/Firmicutes | *Clostridium spiroforme* | 82 | X73441 |
| 555 |  | Clostridia/Clostridiales | *Cloacibacillus* evryensis gen. nov. sp. nov | 90 | CU463952 |
| 598 |  | Clostridia/Clostridiales/Peptostreptococcaceae /Incertae Sedis | *Clostridium lituseburense* strain H17 | 95 | EU887828 |
| 648 |  | Clostridia/Clostridiales/Lachnospiraceae | *Lachnobacterium* sp Wal 14165 | 88 | AJ518873 |
| 766 |  | Clostridia/Clostridiales/Peptostreptococcaceae /Incertae Sedis | *Clostridium glycolicum* stain SN10 | 97 | EU887819 |
| 772 |  | Firmicutes | *Clostridium difficile* 630 | 80 | AM180355 |
| 794 |  | Firmicutes | *Lachnobacterium* sp Wal 14165 | 82 | AJ518873 |

Table S1-S1 continued.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Cluster** | **Phylogenetic affiliation** | **Order/Family/Genus** | ***Genus/Description*** | **% Match** | **Accession No.** |
| 807 | **Firmicutes** | Clostridiaceae/Clostridiaceae 1/*Clostridium* | *Clostridium paraputrificum* G12 | 96 | AY343974 |
| 909 |  | Erysipelotrichi/Erysipelotrichales/Erysipelotrichaceae/*Erysipelothrix* | *Erysipelothrix rhusiopathiae* strain 470/87 | 89 | EF050040 |
| 973 |  | Erysipelotrichi/Erysipelotrichales/Erysipelotrichaceae/*Bulleidia* | *Bulleidia extracta* strain W1365 | 89 | Z36271 |
| 1147 |  | Clostridia/Clostridiales/Incertae Sedis XI/*Sedimentibacter* | *Sedimentibacter* sp MO-SED | 90 | AB598275 |
| 1210 |  | Clostridia/Clostridiales/Ruminococcaceae" | *Ruminococcus* sp YE78 | 86 | KF156793 |
| 1276 |  | Clostridia/Clostridiales | *Clostridiales bacterium* JN18 A24 | 91 | DQ168655 |
| 1351 |  | Clostridia/Clostridiales/Ruminococcaceae | *Ruminococcus* sp C047 | 82 | AB064904 |
| 1356 |  | Clostridia/Clostridiales/Veillonellaceae/*Succinispira* | *Veillonella tobetsuensis* | 86 | AB679110 |
| 1447 |  | Clostridia/Clostridiales/Veillonellaceae | *Veillonella tobetsuensis* | 88 | AB679110 |
| 1655 |  | Clostridia/Clostridiales/Ruminococcaceae/*Ethanoligenens* | *Ethanoligens harbinense* strain CGMCC1152 | 90 | AY833426 |
| 1680 |  | Clostridia/Clostridiales/Veillonellaceae | *Veillonela bacterium* WH074 | 99 | AB298734 |
| 2162 |  | Firmicutes | *Clostridium saccharoqumia* strain SDG-Mt85-3Db | 83 | NR043550 |
| 2389 |  | Clostridia | *Clostridium* sp U40A-5 | 83 | AB059480 |
| 2477 |  | Bacilli/Lactobacillales/Enterococcaceae/*Enterococcus* | *Enterococcus devriesei* strain LMG 13603 | 97 | DQ010644 |
| 2489 |  | Clostridia/Clostridiales/Incertae Sedis XV | *Cloacibacillus evryensis* gen. nov. sp. nov | 90 | CU463952 |
| 2499 |  | Clostridia/Clostridiales/Ruminococcaceae/*Faecalibacterium* | *Fecalibacterium prausnitzii* strian S4L/4 | 84 | HQ457025 |
| 2653 |  | Clostridia/Clostridiales/Clostridiaceae/Clostridiaceae 1/*Clostridium* | *Clostridium* sp | 90 | X95274 |
| 3070 |  | Bacilli/Lactobacillales/Enterococcaceae/*Vagococcus* | *Vagococcus salimoninarium* | 97 | X54272 |
| 3081 |  | Erysipelotrichi/Erysipelotrichales/Erysipelotrichaceae/*Turicibacter* | *Turicibacter sanguinis* strain PC909 | 99 | HQ428099 |
| 3227 |  | Clostridia/Clostridiales/Peptostreptococcaceae /Incertae Sedis | *Clostridium metallolevans* strain SN1 | 94 | EU887815 |
| 3494 |  | Bacilli/Lactobacillales/Carnobacteriaceae 1/*Carnobacterium* | *Carnobacterium* sp BBDP 71 | 93 | DQ337531 |
| 3572 |  | Clostridia/Clostridiales/Veillonellaceae*/Selenomonas* | *Selenomonas ruminantium* | 91 | AB198442 |
| 287 | **Proteobacteria** | Gammaproteobacteria/Xanthomonadales/Xanthomonadaceae | *Pseudoxanthomonas* sp CHNTR 38 | 94 | DQ337597 |
| 359 |  | Alphaproteobacteria | *Sphingopyxis* sp MC1 | 88 | JN940802 |

Table S1-S1 continued

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Cluster** | **Phylogenetic affiliation** | **Order/Family/Genus** | ***Genus/Description*** | **% Match** | **Accession No.** |
| 389 | **Proteobacteria** | Alphaproteobacteria/Rhodobacterales/Rhodobacteraceae/*Amaricoccus* | *Amaricoccus kaplicensis* strain Ben 101 | 98 | NR029201 |
| 397 |  | Alphaproteobacteria/Rhizobiales/Rhizobiaceae/*Kaistia* | *Kaista granuli* strain Ko04 | 94 | NR041362 |
| 469 |  | Betaproteobacteria/Rhodocyclales/Rhodocyclaceae/*Shinella* | *Shinella zoogloeoides* | 99 | AB698675 |
| 485 |  | Alphaproteobacteria/Rhodobacterales/Rhodobacteraceae/*Rhodobacter* | *Rhodobacter* sp TUT3732 | 93 | AB251408 |
| 542 |  | Betaproteobacteria/Burkholderiales/Comamonadaceae/*Rhodoferax* | *Rhodoferax* sp B3 | 98 | DQ268771 |
| 564 |  | Alphaproteobacteria/Rhodobacterales/Rhodobacteraceae/*Rhodobaca* | *Rhodobacter* sp TCRI 3 | 94 | AB017796 |
| 602 |  | Alphaproteobacteria/Rhizobiales | *Methylocella silvestris* BL 2 | 90 | CP001280 |
| 617 |  | Gammaproteobacteria/Legionellales/Coxiellaceae/*Rickettsiella* | *Rickettsiella grylli* | 95 | U97547 |
| 649 |  | Alphaproteobacteria/Rhizobiales | *Oligotropha carboxidovorans* | 90 | AB099659 |
| 727 |  | Alphaproteobacteria/Sphingomonadales/Sphingomonadaceae/*Sphingopyxis* | *Sphingopyxis witflariensis* strain W-50 | 99 | NR028010 |
| 749 |  | Alphaproteobacteria/Rhodobacterales/Rhodobacteraceae/*Rhodobacter* | *Rhodobacter* sp TUT 3732 | 97 | AB251408 |
| 792 |  | Alphaproteobacteria/Rhodobacterales/Rhodobacteraceae | *Rhodobacter* sp EMB 174 | 95 | DQ413163 |
| 983 |  | Alphaproteobacteria/Sphingomonadales/Sphingomonadaceae/*Novosphingobium* | *Novosphingobium hassiacum* strain W-51 | 99 | NR028962 |
| 1046 |  | Alphaproteobacteria/Rhizobiales/Phyllobacteriaceae/*Hoeflea* | *Hoeflea alexandrii* strain AMIV30 | 99 | NR042321 |
| 1123 |  | Betaproteobacteria/Neisseriales/Neisseriaceae/*Uruburuella* | *Uruburuella suis* strain A51 | 99 | HQ259692 |
| 1206 |  | Betaproteobacteria/Burkholderiales | *Herbaspirillum* sp | 91 | AJ012069 |
| 1579 |  | Alphaproteobacteria/Rhizobiales/Hyphomicrobiaceae/*Hyphomicrobium* | *Hyphomicrobium* sp M3 | 97 | AF098790 |
| 1696 |  | Gammaproteobacteria/Pseudomonadales/Moraxellaceae/*Acinetobacter* | *Acinetobacter iwoffii* strain JUN-5 | 97 | KF228924 |
| 1706 |  | Gammaproteobacteria/Pseudomonadales/Pseudomonadaceae/*Flavimonas* | *Pseudomonas* sp HY-14 | 98 | EU620679.2 |
| 2143 |  | Betaproteobacteria | *Rhodoferax ferrireducens* | 90 | AF435948 |
| 2410 |  | Betaproteobacteria/Burkholderiales/Comamonadaceae/*Acidovorax* | *Acidovorax defluvii* strain BSB411 | 93 | NR026506 |
| 2490 |  | Alphaproteobacteria/Rhodobacterales/Rhodobacteraceae/*Paracoccus* | *Paracoccus* sp BBTR62 | 98 | DQ337586 |
| 2592 |  | Alphaproteobacteria/Sphingomonadales/Sphingomonadaceae/*Sphingosinicella* | *Spingosinicella microcystinivorans* | 95 | AB219940 |
| 2823 |  | Alphaproteobacteria/Rhizobiales/Bradyrhizobiaceae/*Bosea* | *Bosea thiooxidans* strain E14 | 98 | AY488508 |

Table S1-S1 continued.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Cluster** | **Phylogenetic affiliation** | **Order/Family/Genus** | ***Genus/Description*** | **% Match** | **Accession No.** |
| 2930 | **Proteobacteria** | Alphaproteobacteria/Rhizobiales/Bradyrhizobiaceae | *Rhodopseudomonas* sp R03 | 99 | EF219053 |
| 3001 |  | Epsilonproteobacteria/Campylobacterales/Helicobacteraceae/*Sulfurimonas* | *Sulfurimonas*sp NS25-1 | 89 | AB175508 |
| 44 | **Bacteroidetes** | Sphingobacteria/Sphingobacteriales | *Myroides* sp XJ193 | 78 | GQ381279 |
| 62 |  | Bacteroidales/Porphyromonadaceae/*Parabacteroides* | *Parabacteroides distasonis* ATCC 8503 | 89 | CP000140 |
| 151 |  | Bacteroidales/Rikenellaceae/*Petrimonas* | *Petrimonas sulfuriphila* strain BSB411 | 94 | NR042987 |
| 362 |  | Bacteroidales/Rikenellaceae/*Petrimonas* | *Proteiniphilum acetatigenes* strain TB 107 | 94 | NR043154 |
| 368 |  | Bacteroidales/Prevotellaceae/*Prevotella* | *Prevotella shahii* strain EHS 11 | 85 | NR024815 |
| 871 |  | Bacteroidales/Porphyromonadaceae/*Parabacteroides* | *Parabacteroides goldsteinii* strain JCM 13446 | 95 | EU136697 |
| 1684 |  | Bacteroidales/Bacteroidaceae/*Bacteroides* | *Bacteroides uniformis* | 99 | AB215084 |
| 1684 |  | Bacteroidales/Bacteroidaceae/*Bacteroides* | *Bacteroides uniformis* strain JCM5828 | 99 | EU136680 |
| 2017 |  | Bacteroidales/Bacteroidaceae/*Bacteroides* | *Bacteroides intestinalis* | 92 | AB437413 |
| 2071 |  | Bacteroidales/Rikenellaceae/*Petrimonas* | *Parabacteroides goldsteinii* | 90 | EU136697 |
| 2157 |  | Bacteroidales/Prevotellaceae/*Prevotella* | *Prevotella* sp BV3C7 | 91 | JN809774 |
| 2157 |  | Bacteroidales/Prevotellaceae/*Prevotella* | *Prevotella veroralis* strain F0319 | 87 | GQ131418 |
| 2170 |  | Bacteroidales | *Bacteroides* sp CS24 | 81 | AB064911 |
| 2259 |  | Bacteroidales/Porphyromonadaceae/*Paludibacter* | *Paludibacter propionicigens* WB4 | 86 | CP002345 |
| 2266 |  | Bacteroidales/Bacteroidaceae/*Bacteroides* | *Bacteroides coprola* strain JCM 12979 | 91 | EU136688 |
| 2274 |  | Bacteroidales | *Parabacteroides* sp NS31-3 | 83 | JN029805 |
| 2464 |  | Bacteroidales/Porphyromonadaceae/*Proteiniphilum* | *Proteiniphilum acetatigenes* strain TB 107 | 84 | NR043154 |
| 2896 |  | Root/Bacteria/Bacteroidetes | *Bacteroides* sp CJ47 | 82 | AB080887 |
| 3226 |  | Bacteroidales/Porphyromonadaceae | *Parabacteroides goldsteinii* strain JCM13446 | 90 | EU136697 |
| 3593 |  | Bacteroidales/Porphyromonadaceae/*Parabacteroides* | *Parabacteroides johnsonii* DSM 18315 strain JCM 13406 | 86 | NR041464 |
| 8 | **Actinobacteria** | Actinomycetales/Micrococcineae/Beutenbergiaceae | *Beutenbergia cavernae* DSM12333 | 91 | CP001618 |
| 212 |  | Coriobacteriales/Coriobacterineae/Coriobacteriaceae/*Eggerthella* | *Eggerthella* sp CJ88 | 88 | AB080902 |
| 281 |  | Actinobacteridae/Actinomycetales/Micrococcineae/Microbacteriaceae/*Leucobacter* | *Leucobacter komagatae* | 95 | DQ083486 |

Table S1-S1 continued.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Cluster** | **Phylogenetic affiliation** | **Order/Family/Genus** | ***Genus/Description*** | **% Match** | **Accession No.** |
| 565 | **Actinobacteria** | Actinomycetales/Micrococcineae/Microbacteriaceae | *Microbacterium* sp RI 2 | 94 | AJ876685 |
| 972 |  | Actinomycetales/Propionibacterineae/Nocardioidaceae/*Friedmanniella* | *Friedmanniella spumicola* strain Ben 107 | 93 | NR024907 |
| 1417 |  | Actinomycetales/Micrococcineae | *Microbacterium* sp BBDP58 | 89 | DQ337517 |
| 1789 |  | Actinomycetales/Propionibacterineae/Nocardioidaceae | *Propionicimonas paludicola* | 92 | AB078859 |
| 2520 |  | Actinomycetales/Propionibacterineae/Nocardioidaceae | *Propionicicella superfundia* strain BL-10 | 94 | NR043609 |
| 3130 |  | Actinomycetales/Corynebacterineae/Mycobacteriaceae/*Mycobacterium* | *Mycobacterium aichiense s*train JS618 | 97 | AF498656 |
| 3154 |  | Actinomycetales/Propionibacterineae/Nocardioidaceae/*Micropruina* | *Micropruina glycogenica* strain Lg2 | 95 | NR024676 |
| 3179 |  | Actinomycetales/Micrococcineae/Microbacteriaceae/*Microbacterium* | *Microbacterium* sp | 96 | JN196543 |
| 3625 |  | Actinomycetales/Micrococcineae/Microbacteriaceae | *Microbacterium thalassium* | 98 | AM943052 |
| 3730 |  | Actinomycetales/Corynebacterineae/Gordoniaceae/*Gordonia* | *Gordonia* sp IFM 0889 | 95 | AB476395 |
| 149 | **Chloroflexi** | Chloroflexi/Anaerolineae | *Levilinea saccharolytica* strain KIBI-1 | 88 | NR040972 |
| 596 |  | Caldilineales/Caldilineacea/Caldilinea | *Chloroflexi bacterium* ET1 | 91 | EU875524 |
| 921 |  | Caldilineales/Caldilineacea/Levilinea | *Longilinea arvoryzea* | 84 | NR041355 |
| 1280 |  | Caldilineales/Caldilineacea/Caldilinea | *Chloroflexi bacterium* ET1 | 85 | EU875524 |
| 891 | **Fusobacteria** | Fusobacteriales/Fusobacteriaceae/*Sebaldella* | *Sebaldella termitidis* ATCC33386 | 99 | NR074413 |
| 2484 |  | Fusobacteriales/Fusobacteriaceae/*Fusobacterium* | *Fusobacterium* sp SRBBR5 | 99 | HM215007 |

sp = species

Table S1-S2 Phylogenetic tree of methanogenic microbial community in anaerobic digestate with closest culturable archaeal relative

*Methanothrix*

Euryarchaeota

Methanobacteria

Methanomicrobia

Methanosarcinales

Methanomicrobiales

Methanobacteriales

Methanocorpusculaceae

Methanosarcinaceae

Methanobacteriaceae

Methanosaetaceae

*Methanobrevibacter*

*Methanocorpusculum*

*Methanosarcina*

*Methanothermobacter*

*Methanosarcina* *bakeri*

*Methanothermobacter* *thermoautotrophicus*

*Methanobrevibacter* *acididurans*

*Methanosarcina* *acetivorans* C2A

*Methanocorpusculum* *sinense*

*Methanosaeta* *concilli* GP6

JN243319

NR074110

NR102903

FR749947

NR028779

NR074260

Class

Phylum

Species

Order

Family

Genus

Accession No.

% Match

98

94

98

99

98

97

Figure S1-S3. Effect of ENPs and bulk metal salts on the abundance and diversity of methanogenic Archaea during anaerobic digestion of sludge in relation to the control. *Methanosarcina* was the only methanogen recovered in the ENPs spiked digester. The resilience, proliferation and population increase of *Methanosarcina* in the ENPs spiked AD within the sampled time indicates that the organism is a nano-tolerant species.

80

40

Similarity (%)

160

190

190

160

190

Treatment / days

Control

ENPs

Metal salts

Anaerobic digestion MDS Plot

Transform: Log (X+1)

Resemblance: S17 Bray Curtis similarity

130

130

160

2D Stress: 0.01

130

Figure S1-S4 2-dimensional NMDS configuration of dissimilarity matrix in anaerobic microbial community at different exposure time and treatments based on 454-pyrosequencing. The numbers (130, 160, and 190) represent the sampling time (days).