Title: Taking the technical microbiome into the next decade

Running title: The technical microbiome

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Originality-Significance Statement

Microbial communities have been determining our lives since the dawn of mankind. It took us centuries to realize their enormous impact, and only in the last decades did we focus on the “microbiome” as an entity, rather than attempting to study microorganisms in pure cultures only. Here, we discussed the, in our opinion, crucial aspects for the coming decade in terms of microbiome management. We discussed two relatively novel concepts within the field of microbial ecology and technology, *i.e.*, phenotyping and synthetic microbial communities. Both concepts will be important to shape research and development in the coming decades to bring microbial technologies to the next level, as we illustrated in the “reversed sanitation” approach to fully maximise the potential of Earth’s microbiome.

Abstract

The “microbiome” has become a buzzword. Multiple new technologies allow to gather information about microbial communities as they evolve under stable and variable environmental conditions. The challenge of the next decade will be to develop strategies to compose and manage microbiomes. Here, key aspects are considered that will be of crucial importance for future microbial technological developments. First, the need to deal not only with genotypes, but particularly with phenotypes is addressed. Microbial technologies are often highly dependent on specific core organisms to obtain the desired process outcome. Hence, it is essential to combine omics data with phenotypic information to invoke and control specific phenotypes in the microbiome. Second, the development and application of synthetic microbiomes is evaluated. The central importance of the core species is a no-brainer, but the implementation of proper satellite species is an important route to explore. Overall, for the next decade, microbiome research should no longer almost exclusively focus on its capacity to degrade and dissipate, but rather on its remarkable capability to capture disordered components, and upgrade them into high-value microbial products. These products can become valuable commodities in the cyclic economy, as reflected in the case of “reversed sanitation”, which is introduced here.

1. Introduction

The origin of life on Earth can be found in microorganisms of the early Archaean period, about 3.8 billion years ago, with the last common universal ancestor (LUCA) (Nisbet and Sleep, 2001; Vlaeminck *et al.*, 2011), although the exact timing and existence of LUCA as sole origin of life remains contested (Steel and Penny, 2010). The diversification of the early-Earth microorganisms in the following eons shaped the planet's biosphere into its current form. The role of microorganisms with respect to everyday human life remains crucial, as they not only influence our health, but they also regulate our basic needs of clean drinking water and nutrition. This nurtured the urge to study microorganisms, which was initially carried out through microscopy by Robert Hooke and Antoni van Leeuwenhoek in the 17th century. Louis Pasteur and Robert Koch made the link with human illness, and succeeded in pure culture cultivations in the 19th century (Wainwright and Lederberg, 1992). In the last decades, research shifted away from this pure culture approach, because microorganisms are not “Einzelgänger”, but host a specific niche in a well-organised microbial community, often containing hundreds of species. The performance of the microbial community strongly depends on the physico-chemical characteristics of their habitat. The importance of the environment as a selective pressure remains a basic rule in microbial ecology (Baas Becking, 1934; de Wit and Bouvier, 2006). Hence, the microbial community should not be considered as a mere assembly of microbial species that happen to be present, but as an interactive entity of microorganisms and physico-chemical parameters, the so-called “microbiome” (Burge, 1988).

Although only in the last decades the “black box” of the microbiome has been (partially) unravelled, related to the advances in high-throughput sequencing techniques (van Dijk *et al.*, 2014), microbiomes have been applied in engineered systems for over a century. The technologies of anaerobic digestion for organic waste stabilization and renewable energy

recovery (McCarty, 1981), activated sludge for wastewater treatment (Ardern and Lockett, 1914), and bio-electrochemical systems (Arends and Verstraete, 2012), among other biotechnological processes, rely on diverse microbial communities, and exist as engineered commodities since the beginning of the 20th century. This is a consequence of the fact that simple physico-chemical parameters, such as pH, conductivity and volatile fatty acid concentrations, are often sufficient for basic (online) process monitoring and control, *e.g.*, in anaerobic digestion (Bjornsson *et al.*, 2000; Boe and Angelidaki, 2012). To evolve towards more in-depth process monitoring, alternative parameters and/or combinations thereof (Madsen *et al.*, 2011; Fdez-Guelfo *et al.*, 2012; Kleybocker *et al.*, 2012) have been applied to enable process stability prediction. However, these approaches, although valuable to monitor process changes, only provide a post-hoc image of the microbiome performance, and merely allow indirect steering.

Direct monitoring of the microbiome in engineered ecosystems can take place by means of amplicon sequencing of the 16S rRNA (gene) for taxonomic identification and/or using the omics approach for activity and functionality measurements. The main challenge, however, resides in the application of this information to steer the microbiome. Bioaugmentation strategies can rely on the introduction of novel species to degrade certain components, such as 3-chloroaniline in activated sludge (Boon *et al.*, 2003), or to introduce more tolerant microorganisms, such as *Methanoculleus bourgensis* in anaerobic digestion (Fotidis *et al.*, 2014). However, the success rate of bioaugmentation is variable, and its effect often limited in time (Goldstein *et al.*, 1985; De Vrieze and Verstraete, 2016).

Here, we discuss three main aspects that can be considered highly important for future sustainable microbiome engineering applications. First, we transcend beyond the borders of taxonomic identification towards the potential importance of phenotypic profiling. Next, the importance of core species and their satellite community in the microbiome is validated

through synthetic microbial community data evaluation. Considering this knowledge, the potential of organized microbiomes for a future and novel approach, *i.e.*, “reversed sanitation” is set forward. The Duke of Wellington was famous for tempting his audiences with the challenge to guess what would be the “other side of the next hill”. In a similar way, we must try to look into the next decade towards the new features and challenges for microbial research to develop advanced microbial technologies, and find out what’s on the other side of the next “microbiome hill”.

2. Engineering of the microbiome

2.1. Beyond taxonomic profiling: phenotyping matters

Phenotypic differentiation, often related to environmental fluctuations, using the same genotype as basis can be observed across the domains of life (Westeberhard, 1989; Kussell and Leibler, 2005; Fusco and Minelli, 2010; Dey *et al.*, 2016). Phenotypic heterogeneity is often a strategy of microorganisms to sustain their activity in dynamic environments (Ackermann, 2015). These phenotypic traits can have a distinct impact on ecosystem performance of which multiple examples already have been described. The acetoclastic methanogen *Methanosaeta harundinacea*, isolated from a full-scale anaerobic sludge blanket reactor (Ma *et al.*, 2006), can shift its morphology between rod-shaped and filamentous, and this process is regulated by means of quorum sensing (Zhang *et al.*, 2012; Zhou *et al.*, 2015). This allows this bacterium to engage granulation (Hulshoff Pol *et al.*, 2004), preventing it from being washed out of the system. The virulence of *Escherichia coli*, for example in the urinary tract (Schreiber *et al.*, 2017), is strongly related to different phenotypes. *Escherichia coli* phenotyping can also play an important role in glucose fermentation, with labour division of the different fermentation steps between different phenotypes (Rosenzweig *et al.*, 1994; Harvey *et al.*, 2014). A similar observation can be made for the closely related strains *Bacillus anthracis*, *Bacillus cereus*, and *Bacillus thuringiensis*, which have very different phenotypes, as they are causing the lethal disease anthrax, food poisoning and a biological pesticide, respectively (Helgason *et al.*, 2000). A more general example of phenotypic plasticity, common for *e.g.*, *Bacillus* and *Clostridium*, is the formation of spores for long-term survival at unfavourable conditions, which by themselves also show a high degree of phenotypic heterogeneity, even within the same bacterial strain (Eijlander *et al.*, 2011).

Whether microorganisms with the same genotype can form different phenotypes strongly determines their mode of action in response to disturbances. Those microbiomes containing

genotypes able to express only a single phenotype solely rely on resistance when exposed to a disturbance. Microbiomes with genotypes that can have multiple phenotypes can rely on resilience and redundancy to sustain ecosystem functioning. For a resistant microbiome, the phenotypes remain unaltered. The main difference between resilience and redundancy lies in the fact that a resilient microbiome returns to its original phenotypic profile after the disturbance is removed, while a redundant microbiome maintains an altered phenotype, even after the disturbance is removed (Allison and Martiny, 2008; Baho *et al.*, 2012; Shade *et al.*, 2012). These different mechanisms are commonly used for taxonomic profiles (Allison and Martiny, 2008), but just as well apply to phenotypic profiles. The main mode of action in response to a disturbance strongly influences the effect of potential corrective measures, hence, monitoring of phenotypic dynamics will be of key importance.

Phenotypic diversity monitoring of microbial communities cannot take place through amplicon sequencing or metagenomics, and requires detailed metatranscriptomic and/or metabolomic studies. Given the time-consuming nature of the omics analysis, alternative approaches need to be developed and optimized (Figure 1). One approach involves the application of a single-cell measurement by means of flow cytometry, which allows to distinguish phenotypic traits (Props *et al.*, 2016). The application potential has been validated for aqueous oligotrophic ecosystems in combination with amplicon sequencing (Props *et al.*, 2018). Application for phenotypic fingerprinting in eutrophic engineered ecosystems, such as activated sludge, anaerobic digestion and mixed culture fermentation, can be considered challenging, due to the complex nature of both the microbiome and the matrix in which it is embedded. Flow cytometry can be used for target-specific monitoring, such as methanogenic activity in anaerobic digestion by means of the autofluorescent F₄₂₀ co-enzyme (Lambrecht *et al.*, 2017). Flow cytometry has been applied directly (Dhoble *et al.*, 2016) or in combination with fluorescent *in situ* hybridization (Nettmann *et al.*, 2013) for microbial community

phenotype dynamics monitoring in anaerobic digestion, landfill leachate (Kinet *et al.*, 2016), or for bacteria integrity detection and faecal indicator monitoring in activated sludge (Foladori *et al.*, 2007). Hence, flow cytometry has the potential to be used for phenotypic fingerprinting in aquatic ecosystems (Wang *et al.*, 2010) and beyond, but, at present, still requires confirmation by alternative techniques, such as amplicon sequencing and conventional plating. An alternative technique with the potential for phenotypic fingerprinting is Raman spectroscopy, which has successful applications in distinguishing phenotypes in pure cultures (Freedman *et al.*, 2016) or for single-cell genomics through cell sorting (Song *et al.*, 2017). Matrix-assisted laser desorption/ionisation – time of flight (MALDI-TOF) mass spectrometry is another technique that can be used to distinguish phenotypes in, for example, cold-stressed *Listeria monocytogenes* (Zhao *et al.*, 2017) and drinking water treatment plants (Sala-Comorera *et al.*, 2016). Hence, the availability of different optimized techniques for phenotypic fingerprinting enables their observation in engineered ecosystems.

The main challenges of phenotypic fingerprinting are related to (1) their application in more complex matrices, due to the diverse microbiome and background noise, and (2) the potential measures to steer the system towards the desired phenotypes in the microbiome. The appearance of potential pathogenic microorganisms (Sahlstrom, 2003; Wen *et al.*, 2009; Kjerstadius *et al.*, 2013) and the proliferation of antibiotic resistance genes (ARGs) (Ju *et al.*, 2016; Guo *et al.*, 2017; Pu *et al.*, 2018) in activated sludge and anaerobic digestion reflects a key challenge for microbial phenotype management. Total elimination of pathogenic and/or ARG-containing microorganisms will result in a “hygienic” end product of the biological process. However, measures taken to eliminate the undesired phenotypes, such as UV treatment, may increase antibiotic resistance, and disturb homeostasis, making the end product susceptible to undesired invasion by other potentially pathogenic species (van Elsas *et al.*, 2012; Van Nevel *et al.*, 2013). Hence, alternative strategies that impose conditions to

avoid virulent phenotypes from becoming metabolically active are needed. This emphasises a key role for phenotypic fingerprinting techniques to not only monitor potential hazards, but also create a platform for targeted remediation strategies to obtain the desired phenotypes. To best of our knowledge, at present, there are no effective methods available to alter or impose the desired phenotype(s) within the microbiome. The least that should be done, is to provide increased attention to reporting the presence of wanted or unwanted phenotypes in the microbiomes of interest. Hence, by complementing the genotypic attention with phenotypic interest, extensive progress in microbiome engineering can be expected (Figure 1).

2.2. Core and satellite species for the Synthetic Community (SynC)

Compared to the amount of literature available on single organisms and diverse microbial communities, only a minor fraction of microbial ecology research makes use of synthetic microbial communities. Synthetic microbial ecology is a collective noun for all assembled ecosystems that are created by a bottom-up approach where two or more defined microbial populations are assembled in a well-characterized and controlled environment (De Roy *et al.*, 2014). These synthetic ecosystems have a lower complexity, higher controllability, higher reproducibility, and are a simplified representation or simulation of natural ecosystems. Synthetic ecosystems can be used (1) to gain insight in fundamental principles, such as metabolic processes, interactions, networking, diversity-functionality relation and nutrient cycling, and (2) to create interactions and communities with desired characteristics and functionality for biotechnological applications.

For microbial technologies, we want to have both very active and very stable communities. Synthetic microbial community (SynC) research has shown that active and stable communities can be obtained by increasing richness (Bell *et al.*, 2005) and evenness (Wittebolle *et al.*, 2009) (Figure 2). Under stress conditions, an invasive species can preserve

indirectly the indigenous functionality, whereas under non-stress conditions the functionality can be threatened (De Roy *et al.*, 2013). This can be considered a remarkable observation, because this could mean that core populations responsible for a specific functionality can be supported by introducing proper “satellite” populations (Figure 2). This hypothesis was examined by combining a methanotroph (core) with a varying richness of heterotrophic bacteria (satellites) (Ho *et al.*, 2014), and the results confirmed that satellites exert a response in core-satellite interaction, resulting in increased activity. This increased activity can be the result of satellites that can remove accumulated inhibitory compounds of the core. Another possibility is that the satellites are producing metabolites that can support the core activity. For instance, based on genomic data, the production of vitamins by the satellites could enhance the methanotrophic core (Stock *et al.*, 2013).

Synthetic microbial communities have, until now, mainly been used to test different ecological theories. The use of these synthetic communities is, however, not restricted to research. Synthetic ecology can be important in the development of specific applications, representing a good balance in terms of complexity, relevance and manageability. A stepwise approach should be applied to design these optimal synthetic communities (Figure 3). First, highly functional enrichments need to be obtained, originating from environments with physico-chemical conditions as close as possible to the ecosystem in which the synthetic microbial community will be applied. Next, the highly active enrichments can be characterised by 16S rRNA gene amplicon sequencing or metagenomics. The metagenomics approach allows better identification and classification of the individual members, and could help in the isolation of the enriched species by examining their distinctive pathways. The next step is the dissection of the enrichment into the individual isolates. These isolates can be further identified by phenotypic and genotypic analysis, and well-suited isolates can be retained to engineer the synthetic communities. For example, isolates containing virulence

factors or antibiotic resistance will be omitted from further use for specific application areas, such as feed and food. The suitable isolates will be combined into synthetic communities, and evaluated for their functionality and long-term stability. Finally, the synthetic communities can be monitored using flow cytometry, real-time PCR, amplicon sequencing or other qualitative/quantitative methods.

Synthetic communities can have specific applications in the circular economy. For example, the European Space Agency (ESA) has designed MELiSSA (Micro-Ecological Life Support System Alternative), a bioregenerative life support system for the complete recycling of gas, liquid and solid wastes during long distance space exploration (Fulget *et al.*, 1999; Hendrickx *et al.*, 2006). Microorganisms play a crucial role in the production of food (cyanobacteria) and in nitrogen cycling (ammonia and nitrite oxidation). This MELiSSA needs to show both high activity and stability to assure long-term functionality (Clauwaert *et al.*, 2017). The engineering of the SynC could assure both functional and compositional stability, and, thus, the engineered synthetic community should be resistant to any perturbation.

Synthetic communities could also play a key role in the industrial fermentation. Bioethanol production, for example, can be considered the core functionality. For ethanol fermentation, alternative sources of sugar are investigated within the second-generation biorefineries, such as lignocellulosic biomass, composed of mainly glucose and xylose (Parajuli *et al.*, 2015; Scoma *et al.*, 2016). However, no efficient microorganisms are known that can convert all sugars into ethanol at high yield. Therefore, synthetic co-cultures with high yields for different sugars could be used. Two-species SynCs of *Zymomonas mobilis* and *Candida tropicalis* have been shown to ferment hydrolysed lignocellulosic biomass into ethanol, with yields up to 97.7% (Patle and Lal, 2007).

The application of synthetic communities for bioremediation can be considered a third promising option. The bioaugmentation with single strains in many cases does not lead to the

desired performance (De Vrieze and Verstraete, 2016). An example is reflected in the complex pathway for linuron degradation (Dejonghe *et al.*, 2003). The core strain *Variovorax* sp. strain WDL1 can use linuron as carbon, nitrogen and energy source. In the enrichments, two satellite strains, *Delftia acidovorans* WDL34 and *Pseudomonas* sp. strain WDL5 were always present, but could not contribute directly to the core activity, *i.e.*, linuron degradation, but both strains were able to remove degradation intermediates. By mixing these strains into a three-species SynC, the rate of linuron degradation improved, due to the synergistic interactions.

A final promising approach is the application of synthetic microbial communities for medical purposes. Microbial faecal transplantations are more and more considered as alternative treatments for several gut-related diseases (Shahinas *et al.*, 2012). However, during these transplantations, there is a potential risk that this “undefined complex community” could harbour pathogens or viruses. An elegant approach can be the use of synthetic communities. For example, a SynC of thirty-three microbial isolates from a healthy donor stool sample has shown the potential of gastro-SynCs to treat *Clostridium difficile* infections (Petrof *et al.*, 2013).

The fact that there is potential for development by applying proper satellite species points to the need of obtaining insight in the various strains that might fit in a particular microbiome. Hence, an extensive list should be created about such putative satellite strains and how they can fit in and complement specific microbial collaborations. Such a list should be screened not only in terms of microbial functionality as such, but must also inform the potential user about the safety status of the organisms and their various phenotypes.

3. The potential of organised microbiomes for reversed sanitation

Current technologies for (waste)water and (semi-)solid organic waste streams treatment, in terms of sanitation, have been focusing on removal and dissipation. In the case of solid and semi-solid organic waste streams, anaerobic digestion is being applied for stabilisation in terms of (1) biodegradable organic matter conversion to the products of “end point fermentation”, *i.e.*, carbon dioxide and methane, and (2) removal of potential pathogens. The removal of potential pathogens, *e.g.*, *Salmonella*, *Escherichia coli*, and *Enterococcus* from manure, slaughterhouse waste or sewage sludge requires either high-temperature anaerobic digestion up to 55-60°C for two hours or longer (Kjerstadius *et al.*, 2013) or a combination of biological and chemical treatment steps (Vanotti *et al.*, 2005). This often requires a high energy investment to obtain end products with a relatively low market value, *i.e.*, methane, carbon dioxide and digestate. A similar approach is being used for high-strength wastewaters, using, for example, the upflow anaerobic sludge blanket technology, through which COD is converted to biogas. While anaerobic treatment allows the recovery of energy through biogas, direct dissipative aerobic treatment of low-strength wastewaters or aerobic polishing of anaerobic effluent constitutes a high energy investment for COD, N and P removal in the order of 40 kWh IE⁻¹ year⁻¹ (Zessner *et al.*, 2010; Verstraete and Vlaeminck, 2011; Meerburg *et al.*, 2015). A similar approach is considered for drinking water purification in which pathogens and residuals removal, often through chemical- or energy-intensive processes, can be considered unsustainable in the long-term. Hence, future microbiome-based technologies will need to move away from mere removal and sanitation, and enable the recovery of high-value products (Figure 4).

A first step towards carbon capture rather than dissipation to carbon dioxide resides in the application of the A-stage of the *Adsorptions-Belebungsverfahren* (AB) process for aerobic wastewater treatment (Boehnke *et al.*, 1997). This A-stage focuses on maximising carbon

storage in a highly biodegradable A-sludge that can be subjected to anaerobic digestion at a COD to biogas conversion efficiency of 85-90% (De Vrieze *et al.*, 2013; Ge *et al.*, 2013; De Vrieze *et al.*, 2015). The application of the A-stage in the full-scale municipal wastewater treatment plant of Nieuwveer, Breda, the Netherlands (Meerburg *et al.*, 2016) and Strass, Austria (Wett *et al.*, 2007) enables up to 30-50% energy savings. Recently, the production of carboxylates through fermentation of A-sludge was investigated to obtain higher-value products, yet, COD conversion efficiencies remained below 30% (Cagnetta *et al.*, 2016). An alternative approach resides in the production of polyhydroxyalkanoates (PHA), using mixed cultures, directly from wastewater in the activated sludge (Salehizadeh and Van Loosdrecht, 2004) or from the fermentation broth (Kleerebezem *et al.*, 2015). Not only A-sludge, but also other liquid and solid waste streams, such as molasses wastewaters (De Vrieze *et al.*, 2016a), primary sludge (Wu *et al.*, 2010), grass (Khor *et al.*, 2016), and food waste (Wang *et al.*, 2014) could be subjected to fermentation instead of anaerobic digestion to obtain higher-value end products. The market value of these different carboxylates ranges from € 600-800 tonne⁻¹ for crude acetate to € 1700-2200 tonne⁻¹ for hydrogen gas (Posada *et al.*, 2013). In contrast, the conversion of 1 tonne of acetate to methane and carbon dioxide, and assuming an electrical efficiency of 40% of the combined heat and power unit, only yields about € 150 (only electricity) to € 250 (electricity and heat) per tonne crude acetate (Deublein and Steinhauser, 2008; De Vrieze *et al.*, 2016a; De Vrieze *et al.*, 2016b). The targeted high-rate production and efficient separation of the carboxylates from the fermentation broth remain important challenges (Agler *et al.*, 2011) that require economic solutions before a sustainable shift from biogas to carboxylate production can be attempted.

An alternative approach concerns the harvesting of the gaseous end products for high-value microbial production. Upgrading of biogas from anaerobic digestion to biomethane (>96 %) for injection in the gas grid represents a first option, but coincides with a power consumption

of 0.05-0.5 kWh m⁻³ raw biogas, depending on the technology (Munoz *et al.*, 2015), and a low industrial market value of natural gas of € 0.25-0.50 m⁻³. A second option resides in the utilization of carbon dioxide from biogas or other point sources to produce carboxylates through electrosynthesis (Nevin *et al.*, 2010; Rabaey and Rozendal, 2010). The current lower production rates, compared to direct carboxylate production *via* fermentation, and subsequent high operational and capital cost, which are a factor 5-10 higher than the actual value of, for example, acetate (Christodoulou and Velasquez-Orta, 2016), do not yet enable economic application of electrosynthesis as such. As a final option, biogas can serve as a carbon and energy source to produce microbial protein as animal feed or even human food (Matassa *et al.*, 2015b; Pikaar *et al.*, 2017). This can be carried out either directly by using methane as carbon and energy source for methane oxidizing bacteria (Strong *et al.*, 2015; Verstraete *et al.*, 2016) or indirectly as energy source to produce electricity for hydrogen gas production through water electrolysis for hydrogen oxidizing bacteria (Matassa *et al.*, 2015a). Although the amino acid content of such microbial protein is similar to conventional protein sources (Matassa *et al.*, 2016), microbial food safety and social acceptance will be important bottlenecks that will determine the future full-scale application potential of microbial proteins and other products that rely on mixed microbial communities. Overall, an integrated approach will be needed in which not only a reversed sanitation approach towards carbon, but also nutrients (mainly N and P) will be essential to guarantee the economic long-term sustainability of microbial technologies for resource recovery.

4. Conclusions

As a key target for the next decades, we must develop the technical side of the microbiomes we strongly depend on. The genomic techniques need be applied in association with major attention to the phenotypic profile of the microbiome. There is an urgent need to develop tools to adequately reveal the phenotypic status of the partners within the functional microbiome, and to establish accurate control strategies. Building stable synthetic microbiomes by implementing proper satellite species next to the core species will be an important strategy, beyond mere understanding, towards implementation of ecological principles. Finally, the most urgent issue for microbial technologies will be to provide better solutions to deal with the waste streams of our industrial society. The present approach is focused on dissipative actions of microbial teams, and the time has come to re-think this approach, and reorient microbial technology towards high-value product recovery and upgrading processes.

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Figures captions:

Figure 1 Schematic overview of the taxonomic and phenotypic fingerprinting of microbial communities for targeted process engineering.

Figure 2 A newly colonized or inoculated ecosystem (1) will show low activity and stability (resistant to environmental changes). Engineered systems (2) have high specific activities, but are sensitive to stress, while natural ecosystems (3) are more resilient, but show less activity. Based on synthetic community research, increasing richness, evenness and the amount of helper bacteria can shape the stable and active engineered microbial processes of the future (4).

Figure 3 Roadmap for synthetic community construction and management.

Figure 4 Schematic overview of the integrated approach for high-value carbon resource recovery from domestic wastewater through reversed sanitation.

Identify “process”

Enriching from different sources

Good performance?

Change enrichment procedure

No

Yes

Metagenome analysis

Isolation of members

Change isolation procedure

All key species isolated?

No

Yes

Genome analysis: members applicable?

No

Select complementary species
from culture collections

Yes

Identify opportunities and assembly of
SynC's

Follow up and management