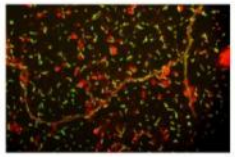
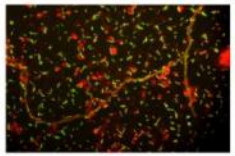



Bioaugmentation in der Umweltbiotechnologie

Heribert Insam
Institut für Mikrobiologie
Universität Innsbruck

University of Innsbruck - Faculty of Biology

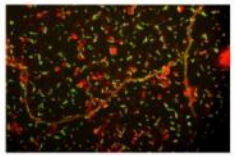
Institute of Microbiology



founded 1669
30.000 students
15 faculties

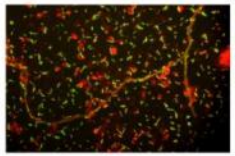
university of innsbruck

Faculty of Biology




→ Die drei Forschungsbereiche:

- Bodenmikrobiologie und Ökophysiologie »
- Mikrobielle Ressourcennutzung »
- Mykologie »




Mikrobielles Ressourcenmanagement »

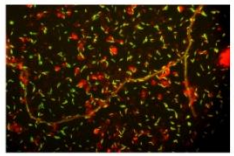
Leiter: Heribert Insam

Angewandte Bodenmikrobiologie
Leiterin: Maria Gomez-Brandon

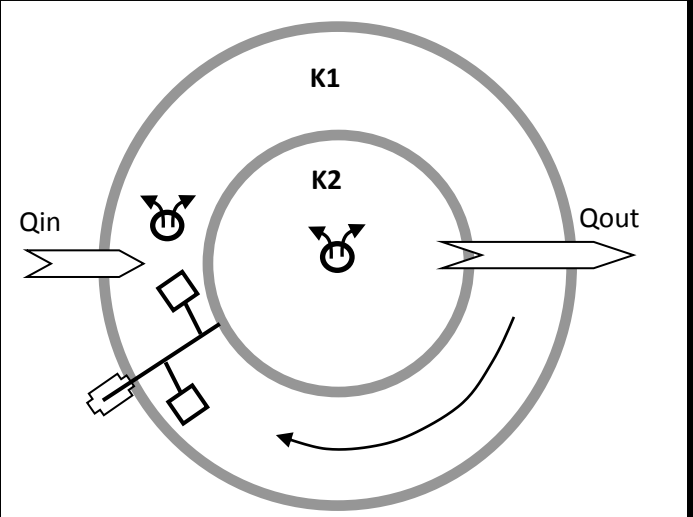
Microarrays
Leiterin: Ingrid Franke-Whittle

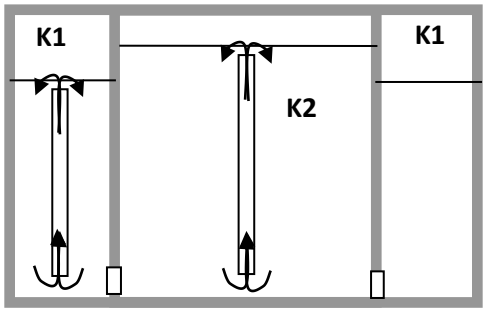
Biogas Technologie
Leiterin: Sabine Podmirseg

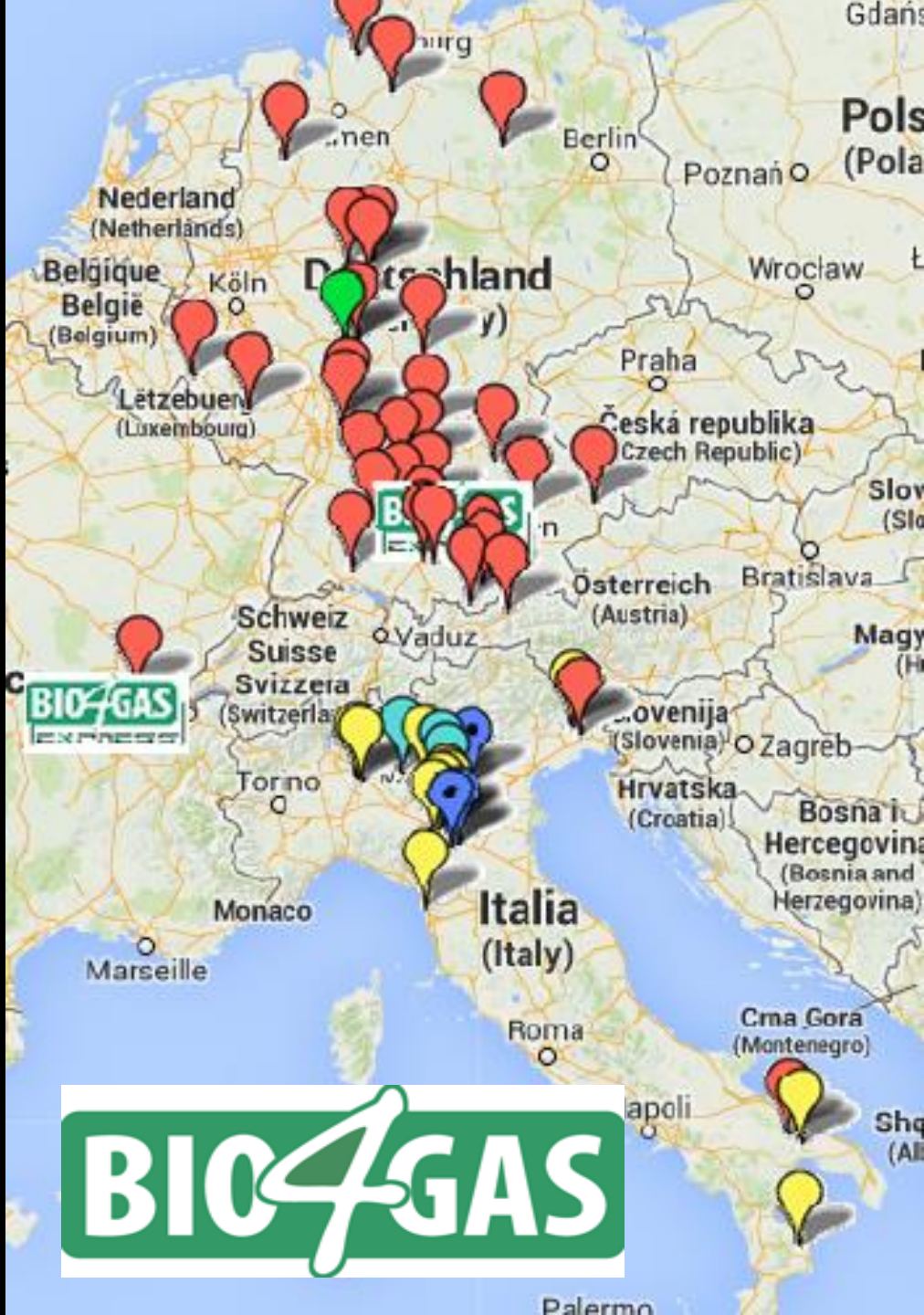
Abwasser
Leitung: Thomas Pümpel und Sabine Podmirseg



- Prozessverbesserung (z.B. durch Anaerobe Pilze) (FFG + HRSM)
- Entwicklung von Biogasanlagen (BIO4GAS GmbH spinoff)
- Biogaspotential diverser (exotischer) Substrate
- Gasreinigung (BioGap) - BoKu
- BioGasMonitoring (MOST-Projekt) + Kleinprojekte mit Syneco (Italien)


K1
K2
Qin
Qout


K1
K1
K2

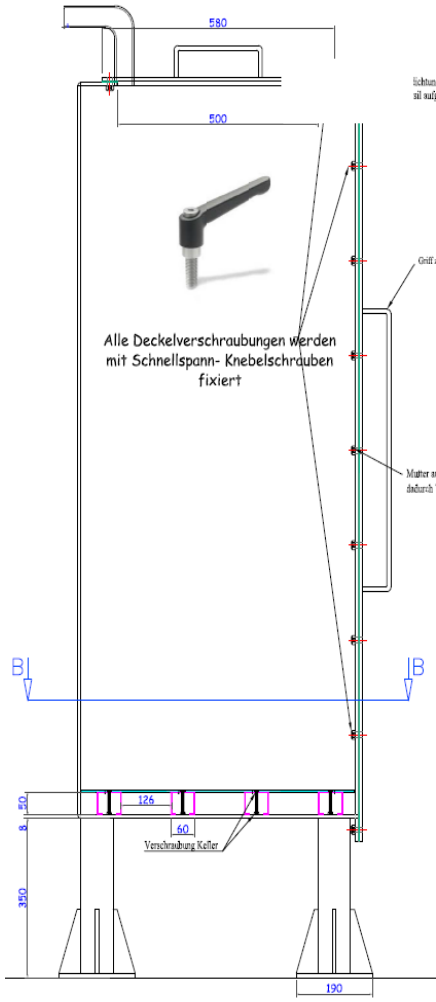

Nederland
(Netherlands)
Belgique
België
(Belgium)
Köln
Berlin
Poznań
Wrocław
Praha
Česká republika
(Czech Republic)
Lëtzebuerg
(Luxembourg)
Österreich
(Austria)
Bratislava
Schweiz
Suisse
Svizzera
Vaduz
Slovenija
(Slovenia)
Zagreb
Hrvatska
(Croatia)
Bosna i
Hercegovina
(Bosnia and
Herzegovina)
Torino
Monaco
Marseille
Italia
(Italy)
Roma
Crna Gora
(Montenegro)
BIO4GAS

BioGAP: Biogasreinigung mit Holzaschefilter zur CO_2 und H_2S -Eliminierung **FFG**

BioTreaT: Kompetenzzentrum für mech/biol Abwasser und Abfallbehandlung (**TSA**)

Biogasmonitoring

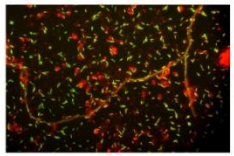
Koordination eines interdisziplinären Teams aus Ingenieuren/Naturwissenschaftern (Biologen, Bauingenieure, Umwelttechniker, Chemiker)



Aktuell: Machbarkeitsstudie INSIDE, Sickerwasserproblematik aus Deponien **(FFG);** Partner: ATM, BFW, IUT

Gründung des Spinoffs **BIOTreaT GmbH**

BioTreaT

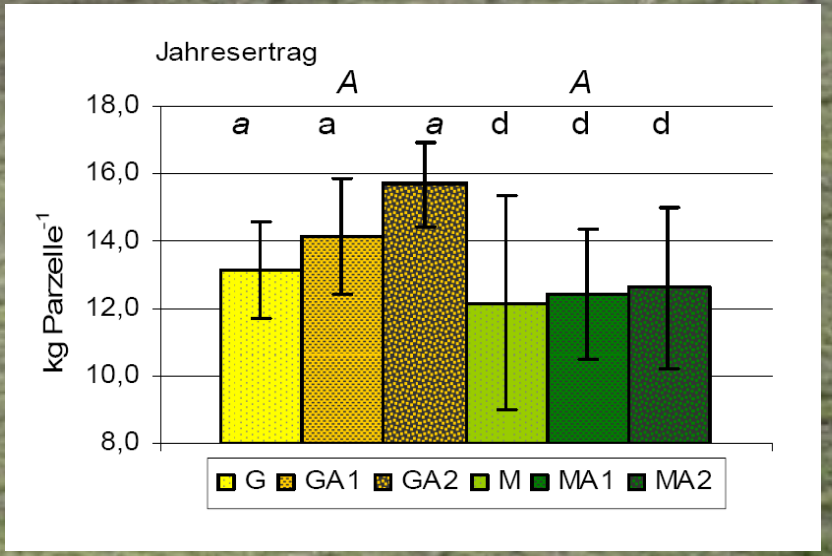



Soil and Compost

- Verwertung von Reststoffen
  - Dünger aus Holzaschen
  - Nutzung von Gärresten
  - Kompostierung
- Biolog. Abbau von Totholz → Bodenbildung (FWF)
- Phosphorverfügbarkeit (Koop. Kemusadewi, Wayan-Bali/Indonesien, Univ. Rostock, Projekt Donauanrainerstaaten)
- Bodenmüdigkeit im Obstbau
- Boden-Chronosequenzen

Gärrest und Asche

Alexander Knapp
Marina Fernandez
Sebastian Hupfauf



Apple Replant Disease - BioIncrop

- Bekannt von vielen Apfelregionen weltweit
- Etiologie : verschiedene biologische Faktoren verantwortlich

Ziel: Verbesserung des Wissens um die Bedeutung der Boden-Mikroflora (e.g. plant disease suppressiveness)

Experimente: Glashaus- und Feldversuche in D, CH, I, A mit unterschiedlichen organischen Zusätzen, u.a. Komposte

Methoden: 16S rRNA basiertes Pyrosequencing von kanken und gesunden Böden, mikrobielle Biomasse/Basalatmung, COMPOCHIP-Analysen der Komposte



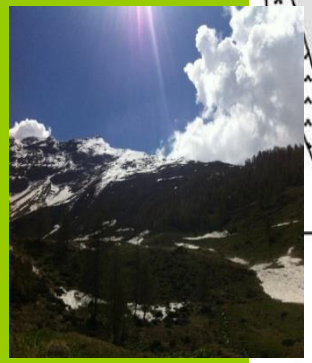


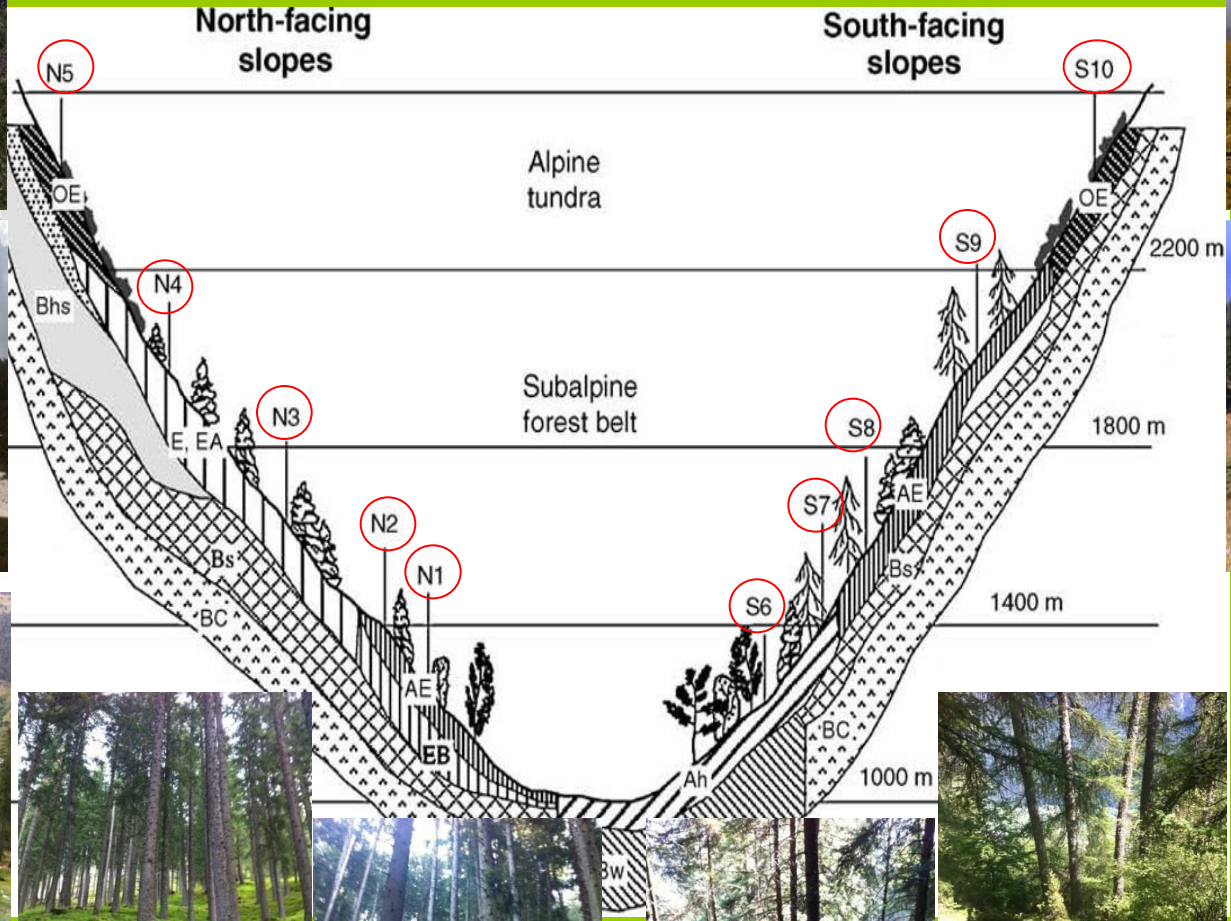

Argentinien-Patagonien

Glaciar Hielo Azul

D.A.CH. – DecAlp Project

Effect of climate on coarse woody debris decay and incorporation into the soils of forested Alpine areas



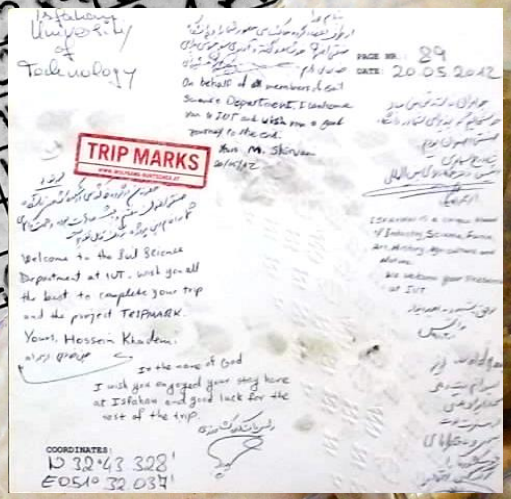


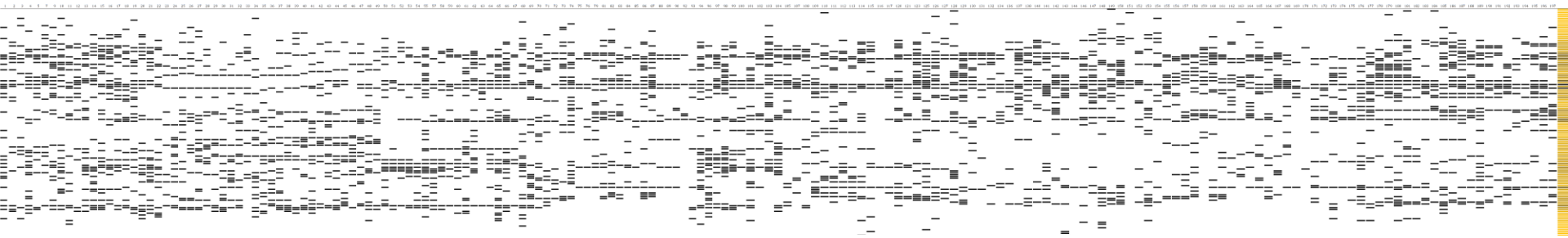
STUDY AREA: Val di Rabbi (Trentino)

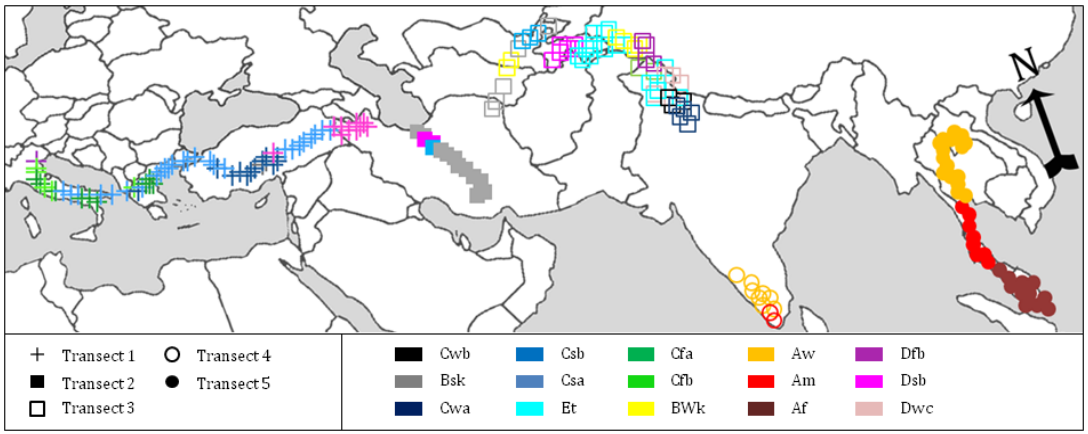
Egli et al. (2006)

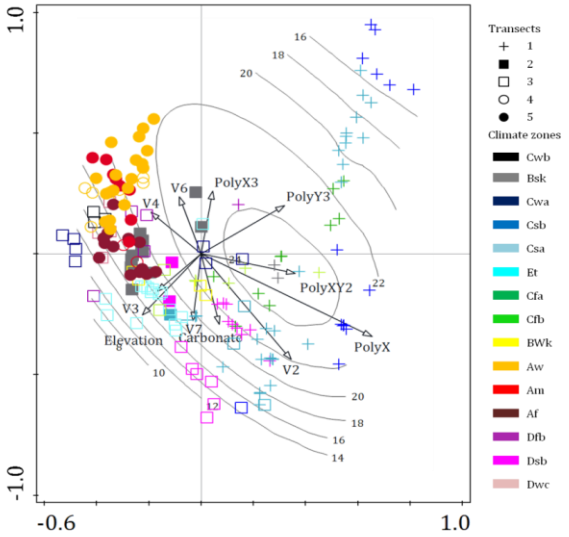
TRIPMARKS

is everything everywhere?

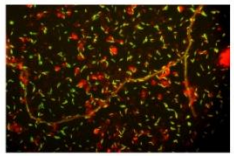




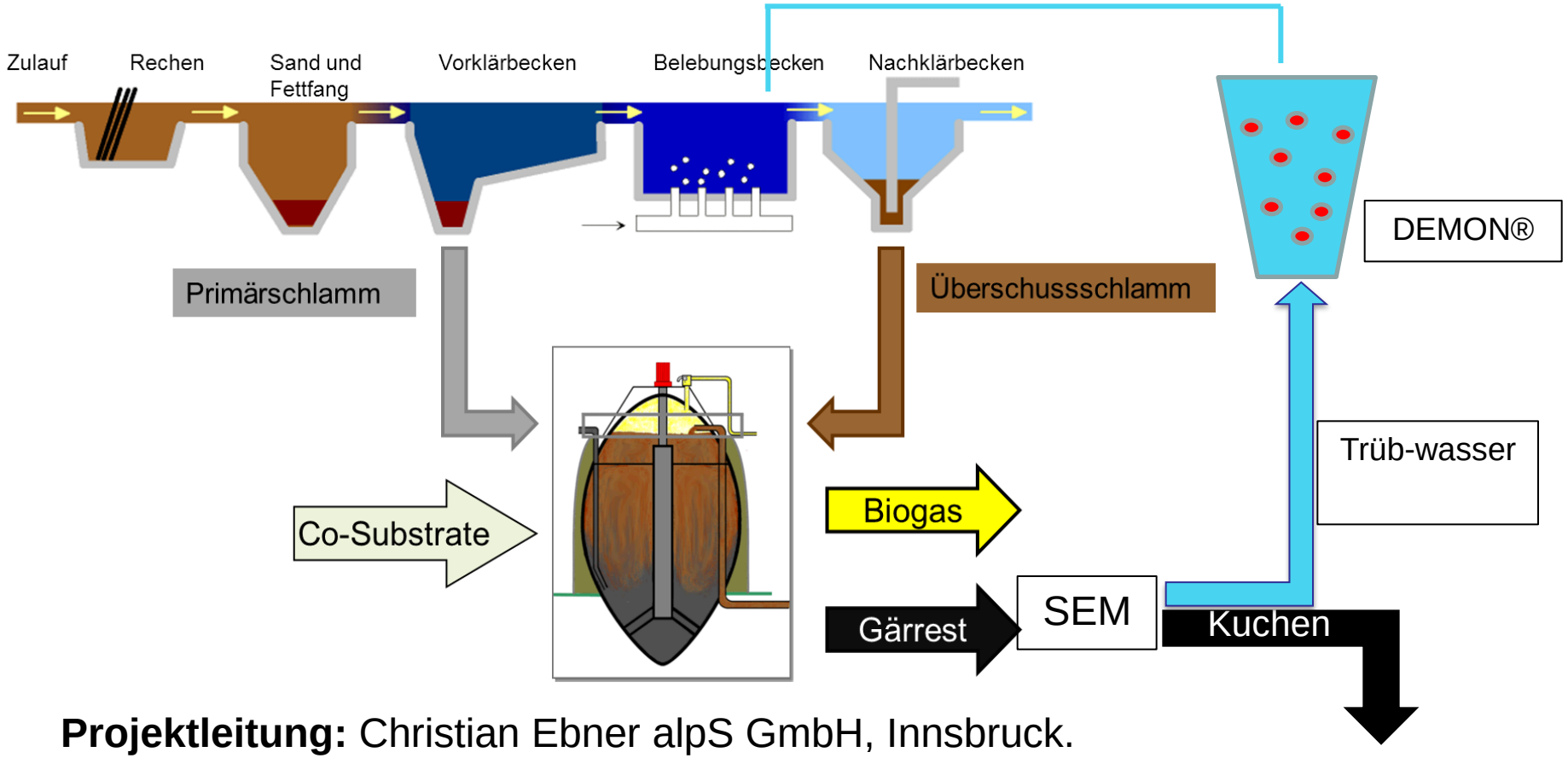




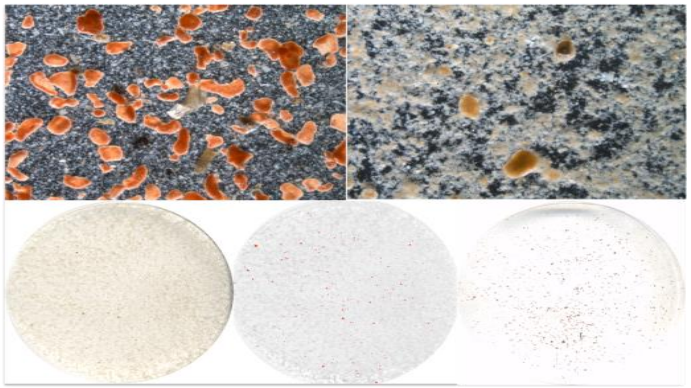
ARAFerm

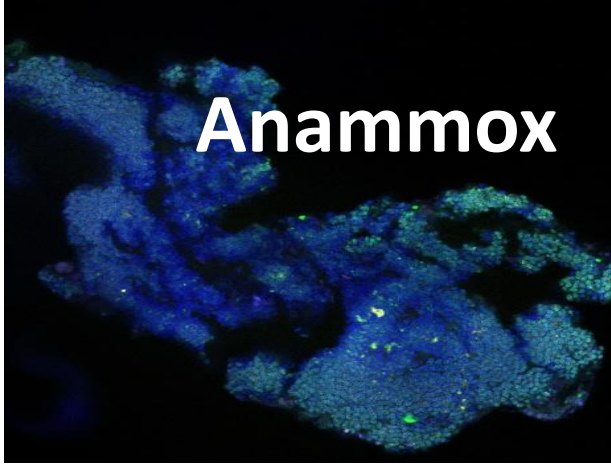


Optimierte Faulraumnutzung incl. Filtratwasserbehandlung



Projektleitung: Christian Ebner alpS GmbH, Innsbruck.
Uni Innsbruck: Thomas Pümpel, M. Kuprian, S. Waldhuber
Kooperation mit Inst. f. Infrastruktur: A. Bockreis, W. Müller, A. Wörle





Analytik
- Molekularbiologisch
- Physiol. Aktivitäten
- Inhaltsstoffe (Häm)

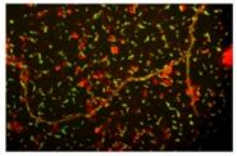
Grundlagenarbeiten
- Spurenelementbedarf (Fe)
- Inhibierungen
- …

Labor- und Pilotanlage DEMON®
- Optimierung Laboranlage
- Erweiterung Pilotanlage

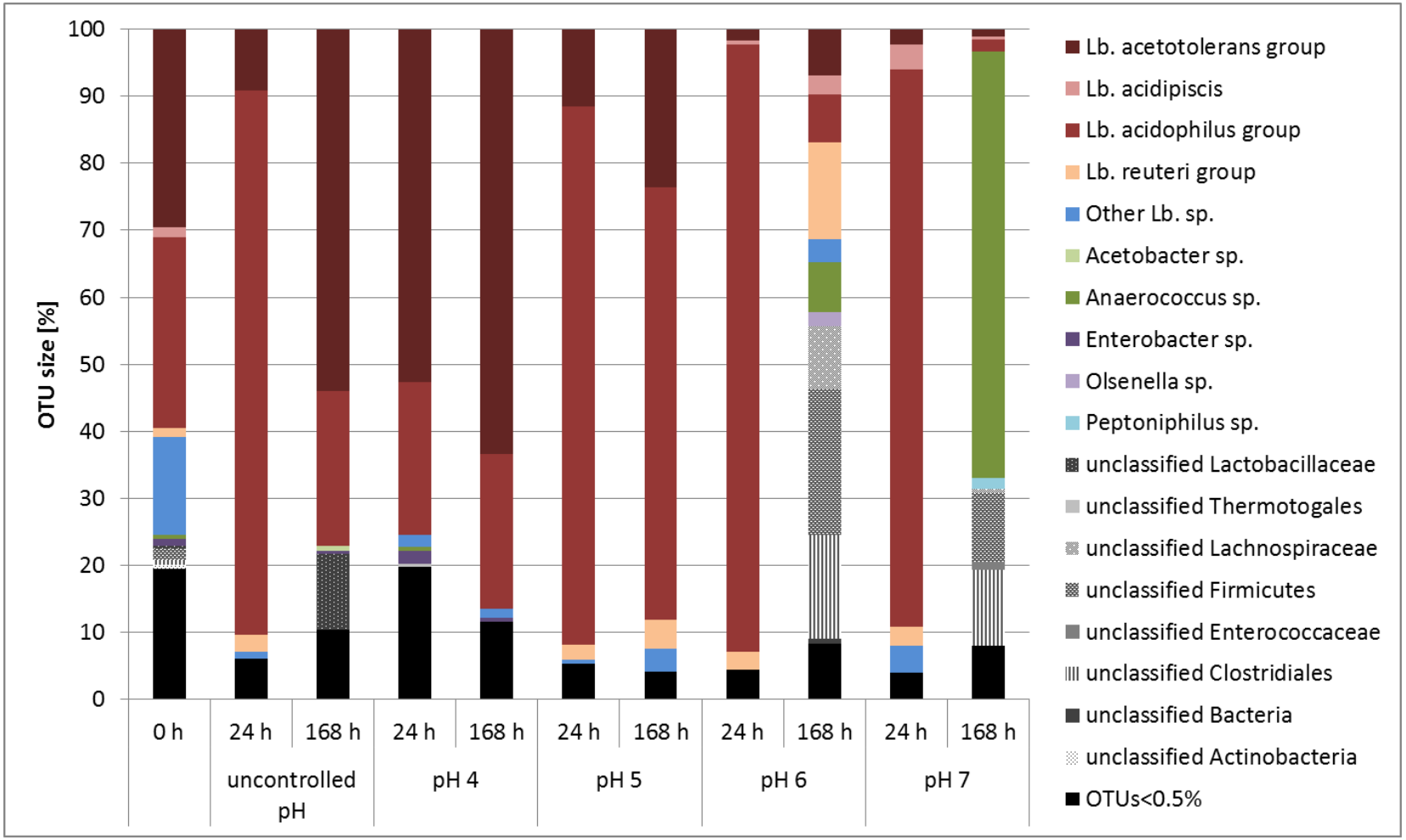
Störfallanalysen DEMON®
- Einfluss Organik (org. Sren)
- Salz (osmotischer Druck)
- Feststoffeintrag
- …

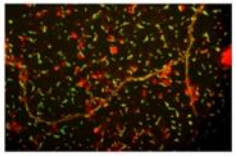
Neue Anwendungen DEMON®
- Industrie-Abwasser
- Deponie-Sickerwasser
- Trübwasser ldw-Biogasanlagen
- Brüdenkondensate
- …

Milchsäuregeärung von Bioabfall



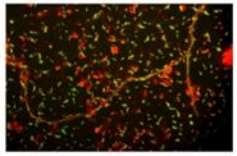

Selektive Anreicherung von Lactobacilli als Produzenten von Laktat






Lourens Baas Becking (1931).
Gaia of Leven en Aarde. M. Nijhoff, The Hague

- Mikrobieller Kosmopolitanismus
- Passive Verbreitung
- Nischenokkupation
- Adaptation

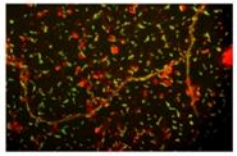



Bioaugmentation *Bio (gr. Leben)* and *augmentatio* (verbessern)

Beschleunigung durch biologischen Umbau/Abbau durch Zugabe von (Mikro)organismen

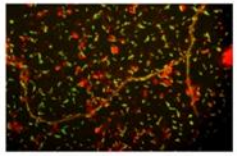
Alle drei Domänen des Lebens: Bakterien, Archaeen, Eukaryoten (Pilze)

Reinkulturen, Mischkulturen oder ganze (in der Zusammensetzung nicht bekannte) mikrobielle Gemeinschaften (EM®; Schlämme, Böden, Sedimente)




Bioaugmentation

- Hydrolyse bei der Anaeroben Vergärung (Biomethanisation)
  a. Aerob b. Anaerob
- Methanogenese
- Nitrifikation in der Abwasserreinigung
- Anaerobe Ammoniumoxidation in DEMON®
- Kompostierungsprozesse




Bioaugmentation

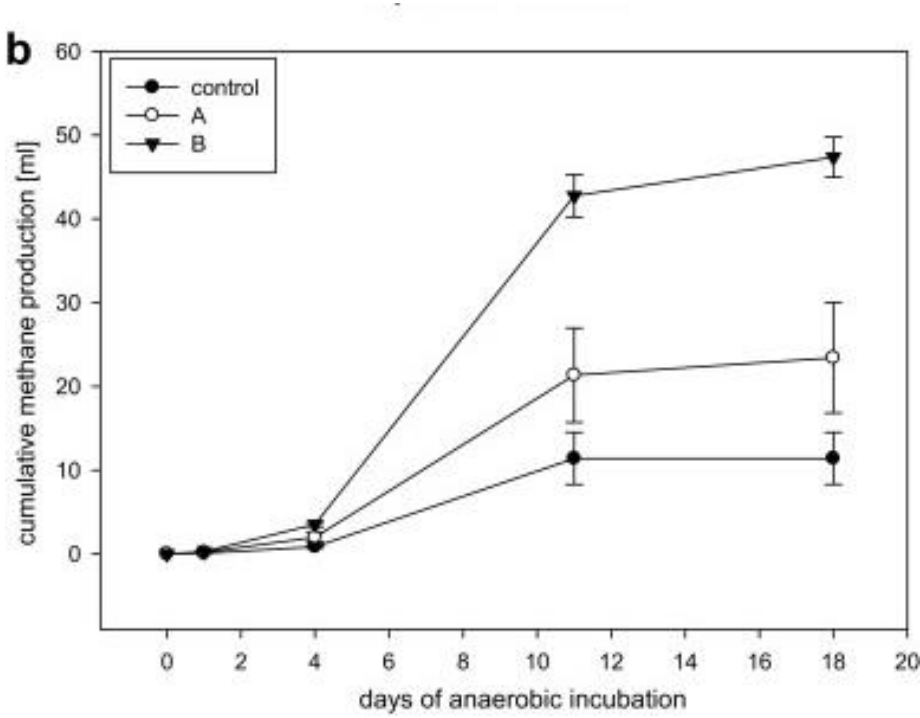
- Hydrolyse bei der Anaeroben Vergärung
  a. Aerob b. Anaerob → **Pilze**
- Methanogenese bei der Anaeroben Vergärung → **Archaea**
- Nitrifikation bei der Abwasserreinigung → **Bakterien**
- Anaerobe Ammoniumoxidation in DEMON® → **Bakterien**
- Kompostierungsprozesse → **Bakterien**

Trichoderma viride im Rahmen einer aeroben Vorinkubation führte zu einer signifikanten Steigerung der Gasproduktion aus Bioabfall.

→ Erhöhte Cellulase-Aktivität
→ Bessere Substratverfügbarkeit für die anaeroben Mikroorganismen
→ Verminderte Acetatwerte
→ Keine Abreicherung verwertbarer Substrate
→ Hohes Praxispotential

Methanbildung innerhalb von 18 Tagen anaerober Inkubation
Kumulative Methanproduktion

- **Kontrollle**
- **A: 5 % *T. viride***
- **B: 15 % *T. viride***



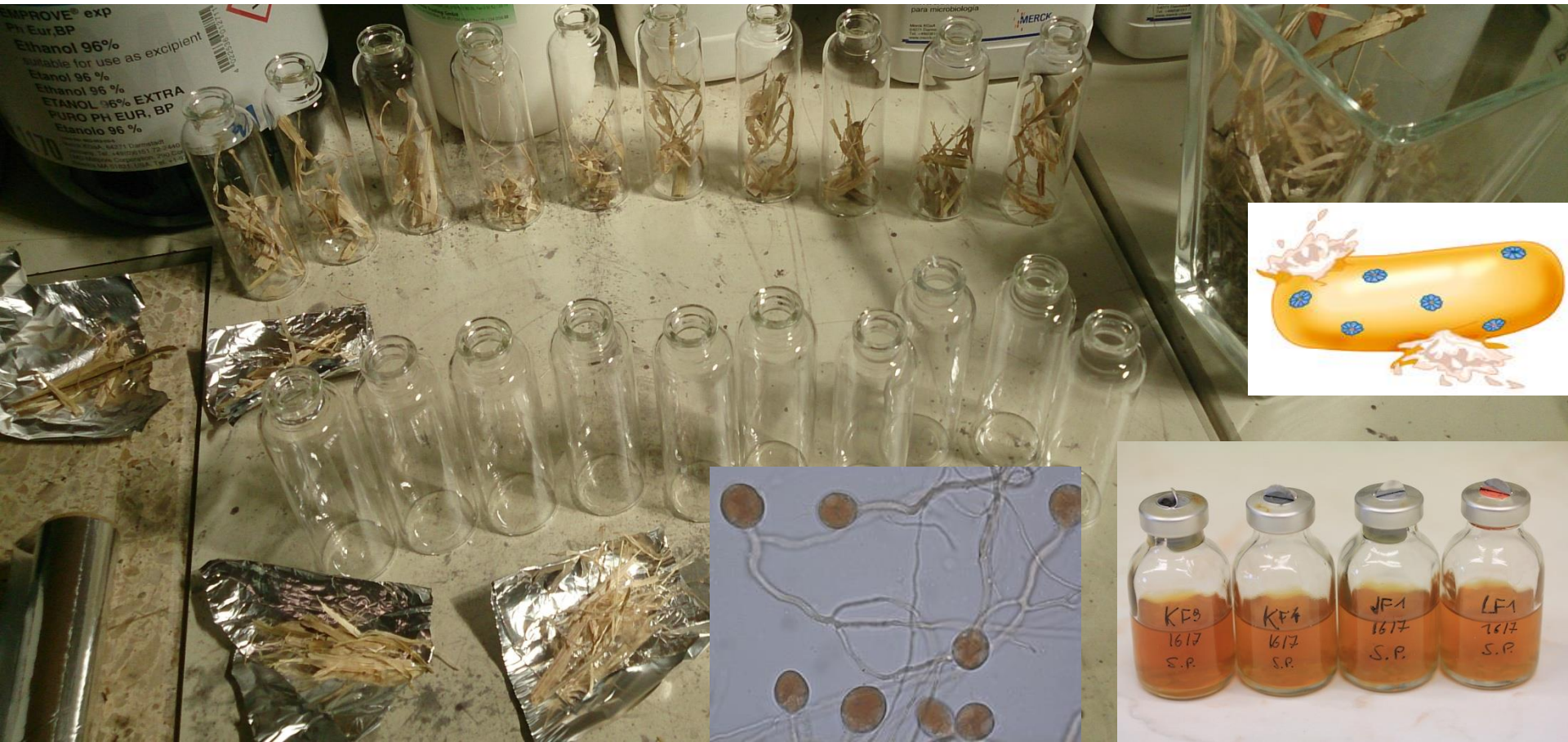
Wagner, Schwarzenauer, Illmer, 2013, Improvement of methane generation capacity by aerobic pre-treatment of organic waste with a cellulolytic *Trichoderma viride* culture. J. Env. Manage. 129, 357 - 360

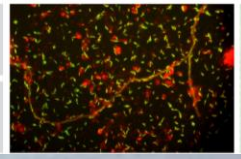
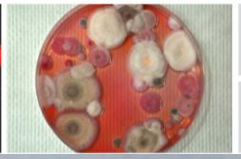
HRSM - MicrobeEnergy

Neocallimastigomycota – culture collection

Vorbehandlungsstrategien für Biomethanisierung von cellulosehaltigen Substraten

Kooperation mit FH Wels/MCI, Diss Magdalena Nagler, MSc: Bettina Nothegger, Koop mit Ursula Peintner, Philipp Dresch

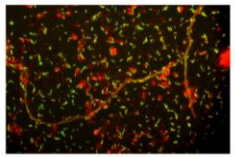
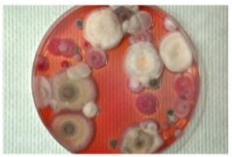


Anaerobe Pilze

1. Isolierung aus Faeces vom Steinbock (*Capra ibex*)
2. Molekulare Charakterisierung
   - PCR, Cloning, Sequencing =>phylogenetic assignment
   - Acid Lactofuchsin Staining
3. Assoziierte Methanogene
4. Stammselektion
   - Microarray, FISH => methanogen
5. Biogaspotential (AMPTS)

   inoculation of biogas reactors with active vs. dead fungal cultures

Methodology

active

Autoclave

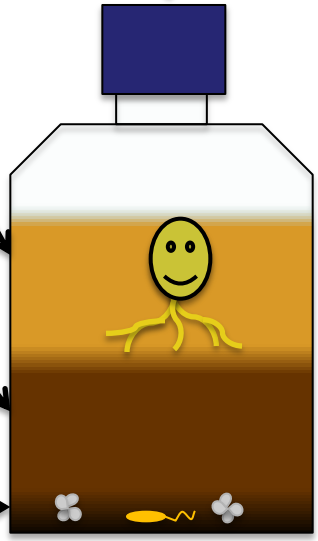
sterilized

- 3 weeks
- gas prod.
- substrate degradation
- physico-chemical & molecular analysis

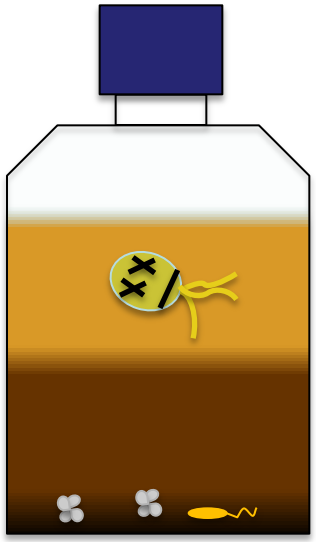
200 mL fungal pre-cultures (2 d)

170 mL sterile cattle manure and straw = substrate

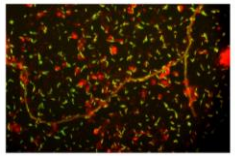
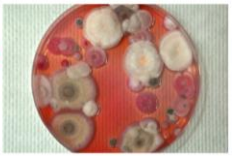
30 mL unsterile cattle manure (active microbial consortium)



treatment



control

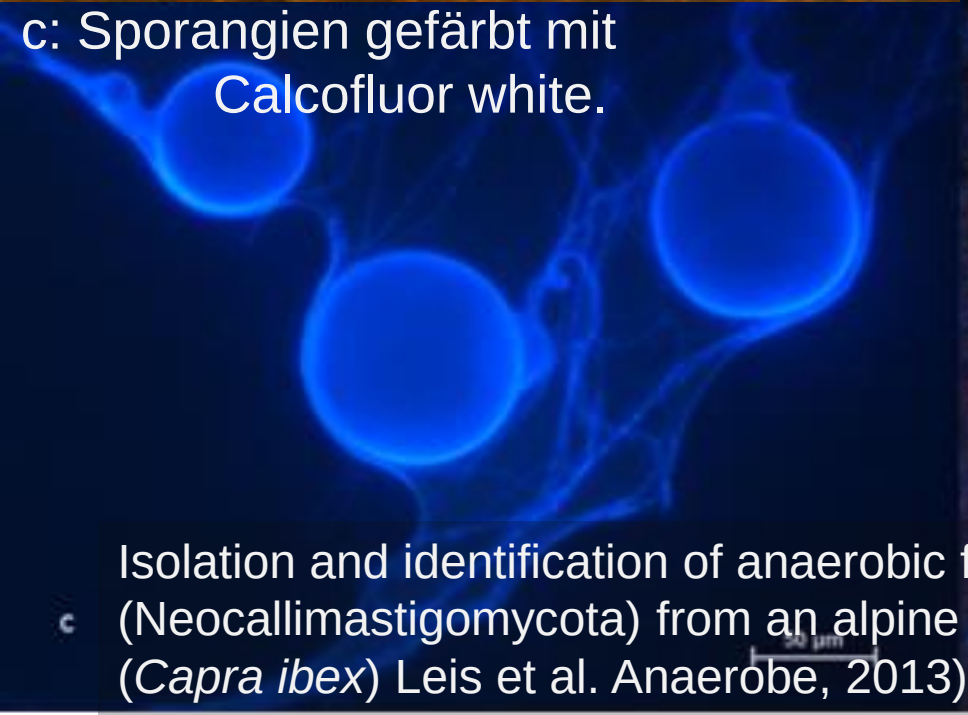



Methodology

assessing activity via in-growth meshbags

- 53 µm mesh width allows entrance of fungi while keeping substrate inside
- microscopic analysis of content after incubation

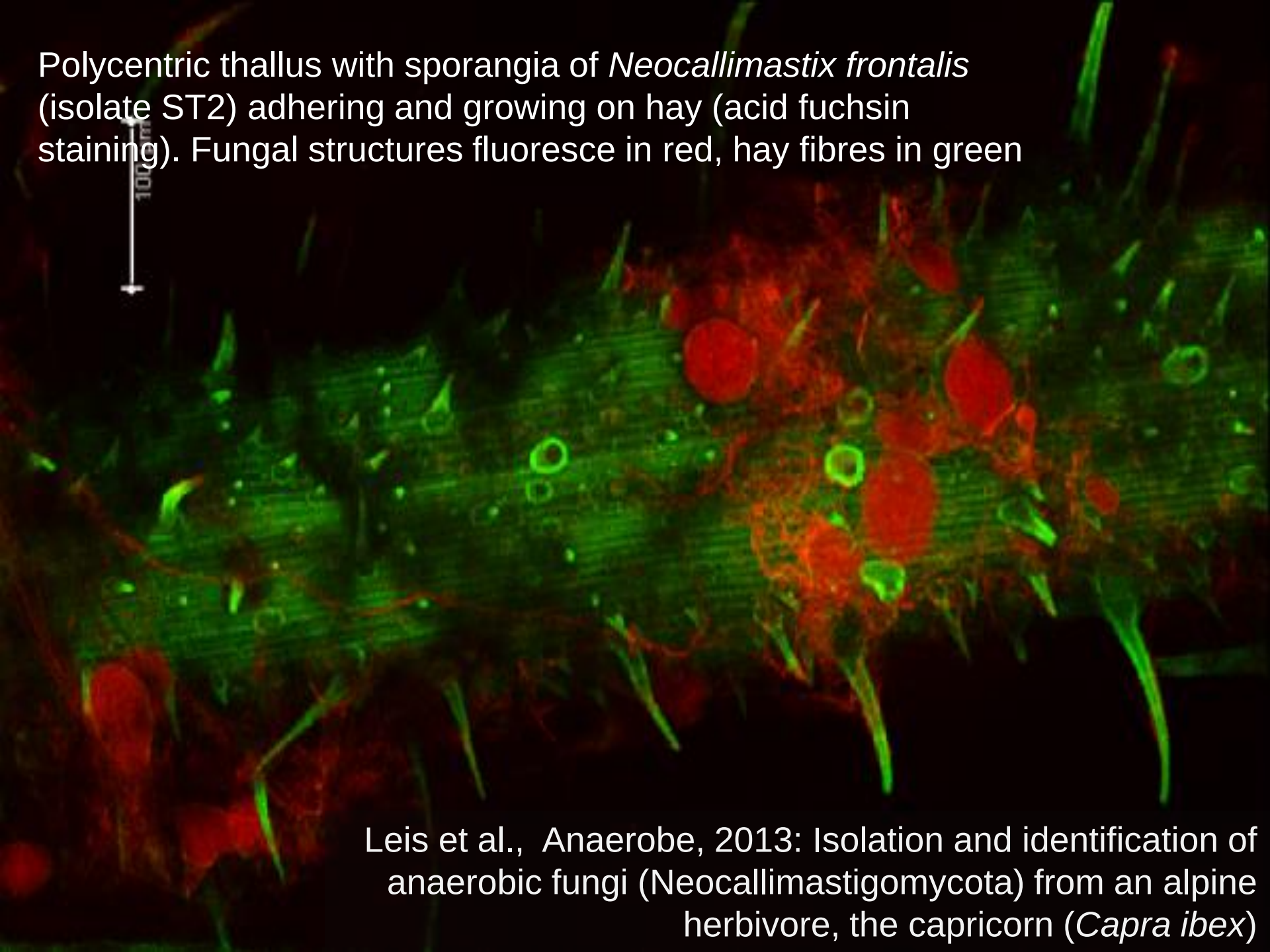
Neocallimastix frontalis, a-b: Myzel und Sporangien in verschiedenen Wachstumsstadien, Lichtmikroskopisch).



c: Sporangien gefärbt mit Calcofluor white.

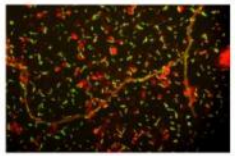
d: gefärbt mit DAPI, Zellkerne in den Sporangien und im Myzel

Isolation and identification of anaerobic fungi (Neocallimastigomycota) from an alpine herbivore, the capricorn (*Capra ibex*) Leis et al. Anaerobe, 2013)



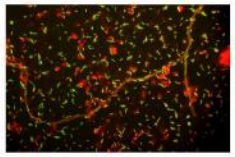
Polycentric thallus with sporangia of *Neocallimastix frontalis* (isolate ST2) adhering and growing on hay (acid fuchsin staining). Fungal structures fluoresce in red, hay fibres in green

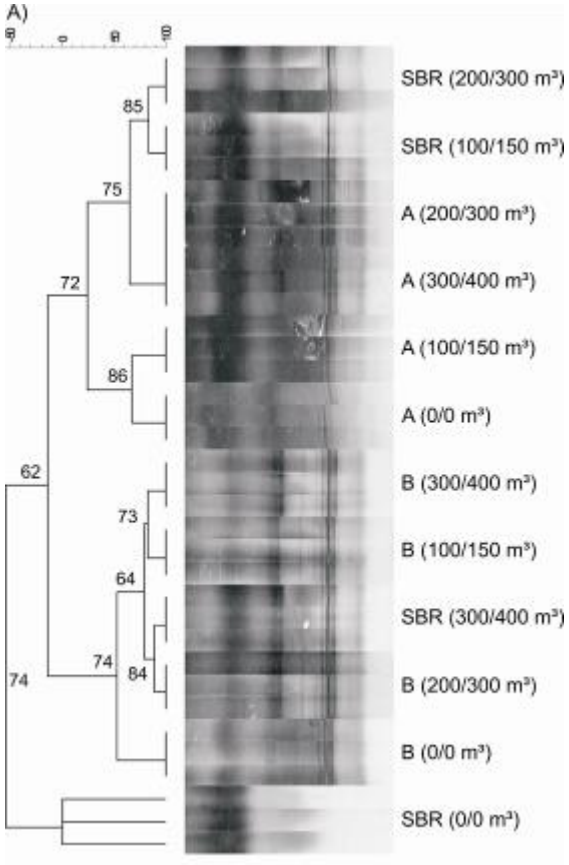
Leis et al., Anaerobe, 2013: Isolation and identification of anaerobic fungi (Neocallimastigomycota) from an alpine herbivore, the capricorn (*Capra ibex*)




…bis jetzt noch keine schlüssigen Ergebnisse zur Steigerung der Hydrolyse

Hauptproblem ist die schwache Kultivierbarkeit

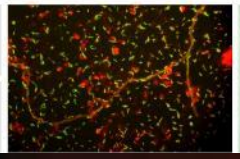


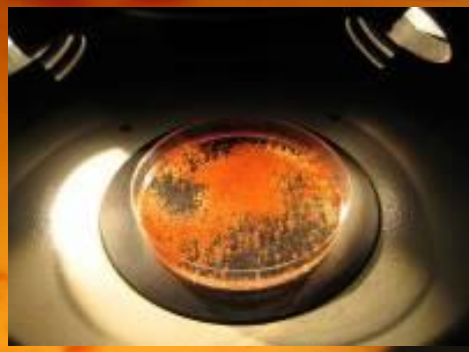
Bioaugmentation von Nitrifikanten in einer zweistufigen Abwasserreinigung

→Stickstoffentfernung verbessert
→Energiebedarf reduziert
→Vermeidung von Erweiterungs-bauten der Kläranlage

Podmirseg et al. (2014) *Water Sci Technol*

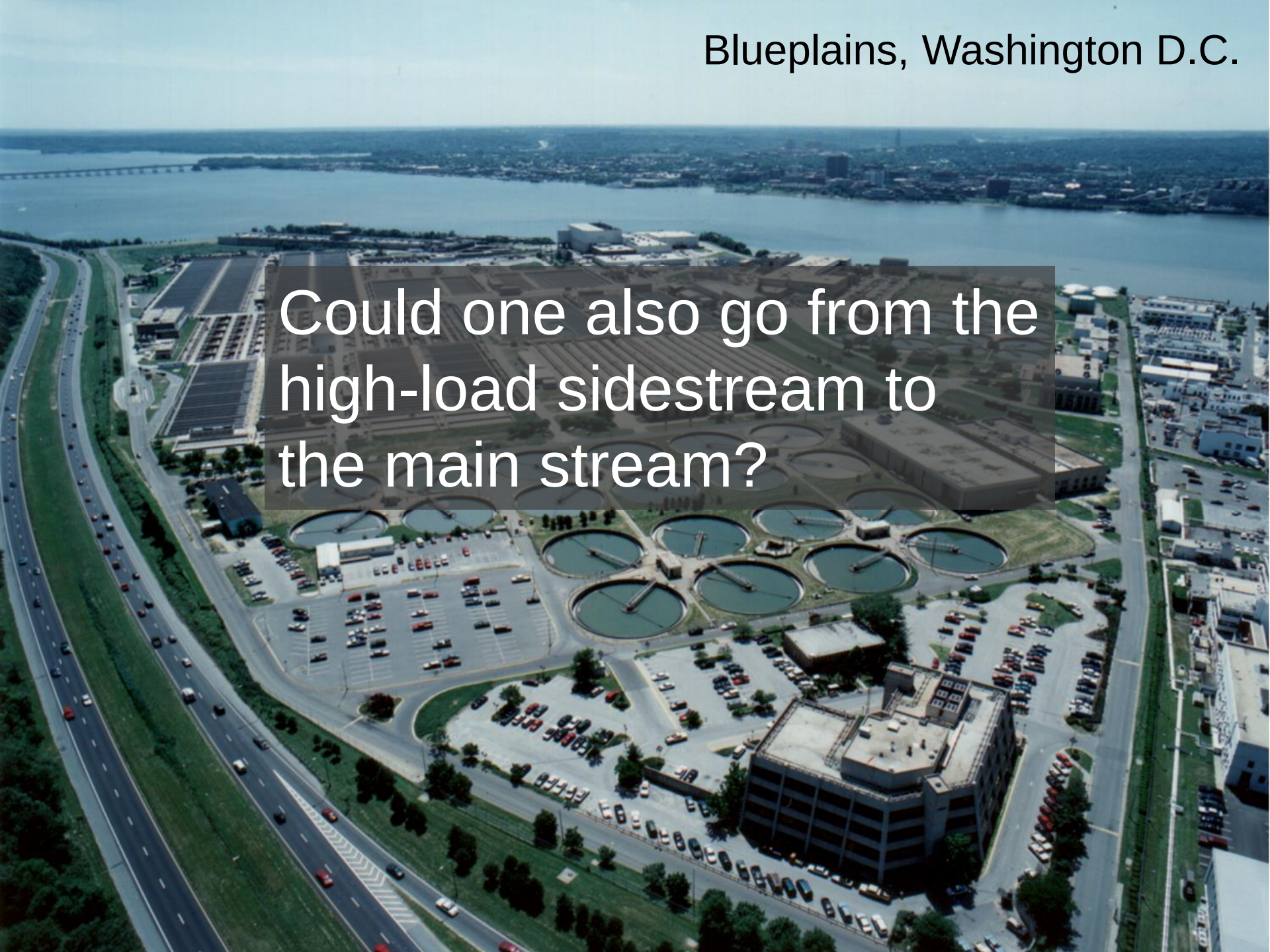


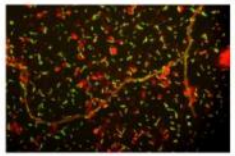
ANAMMOX Bacteria in Sludge Granules of a DEMON® Deammonification Plant



- With DEMON® anammox is used at an industrial scale → First wastewater treatment plant that produces more energy than it needs
- pH regulated process
- Granules show specific community composition
- Granule separation enables effective re-inoculation

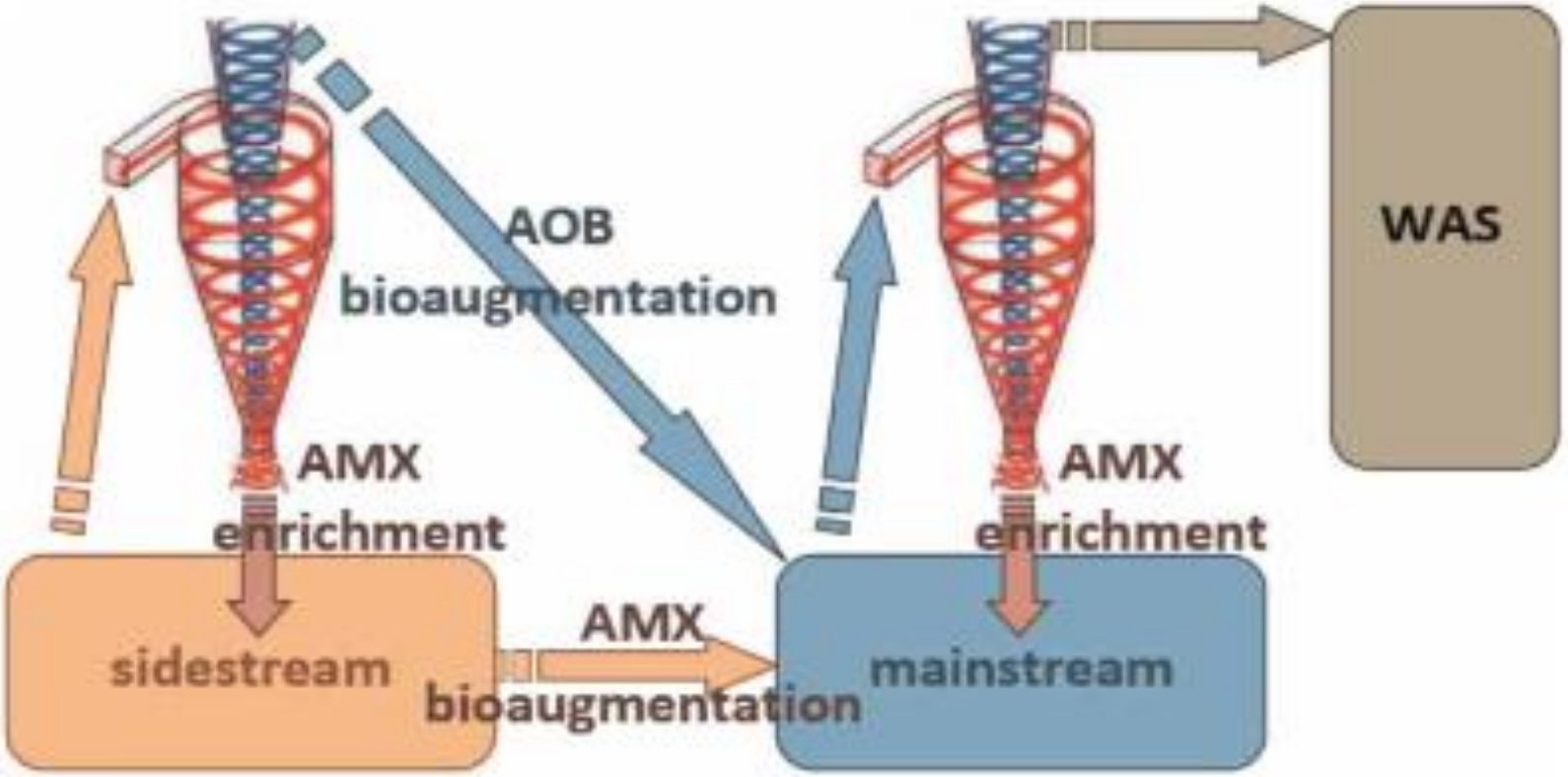
Podmirseg et al., 2014 Water Research


Blueplains, Washington D.C.
Could one also go from the high-load sidestream to the main stream?



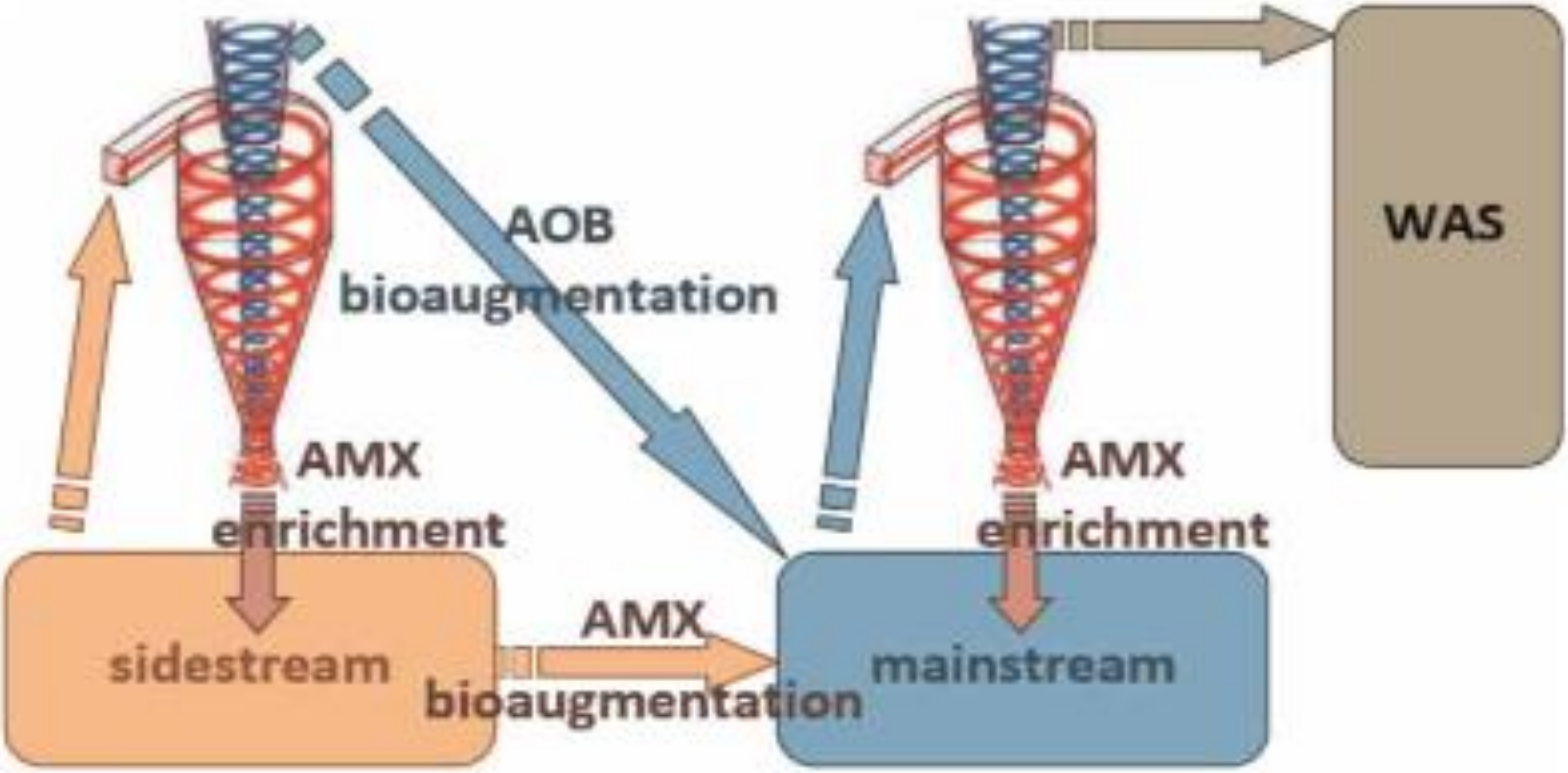

- Dichtebasierte selektive Anreicherung der Anammox-Granulen

- Intermittierende Belüftung um Nitritoxidierer zu reprimieren →Maximale Wachstumsrate der NOB muss heruntergesetzt werden um deren Konkurrenz zu den Anammox einzuschränken (Reduktion des gelösten Sauerstoffs)

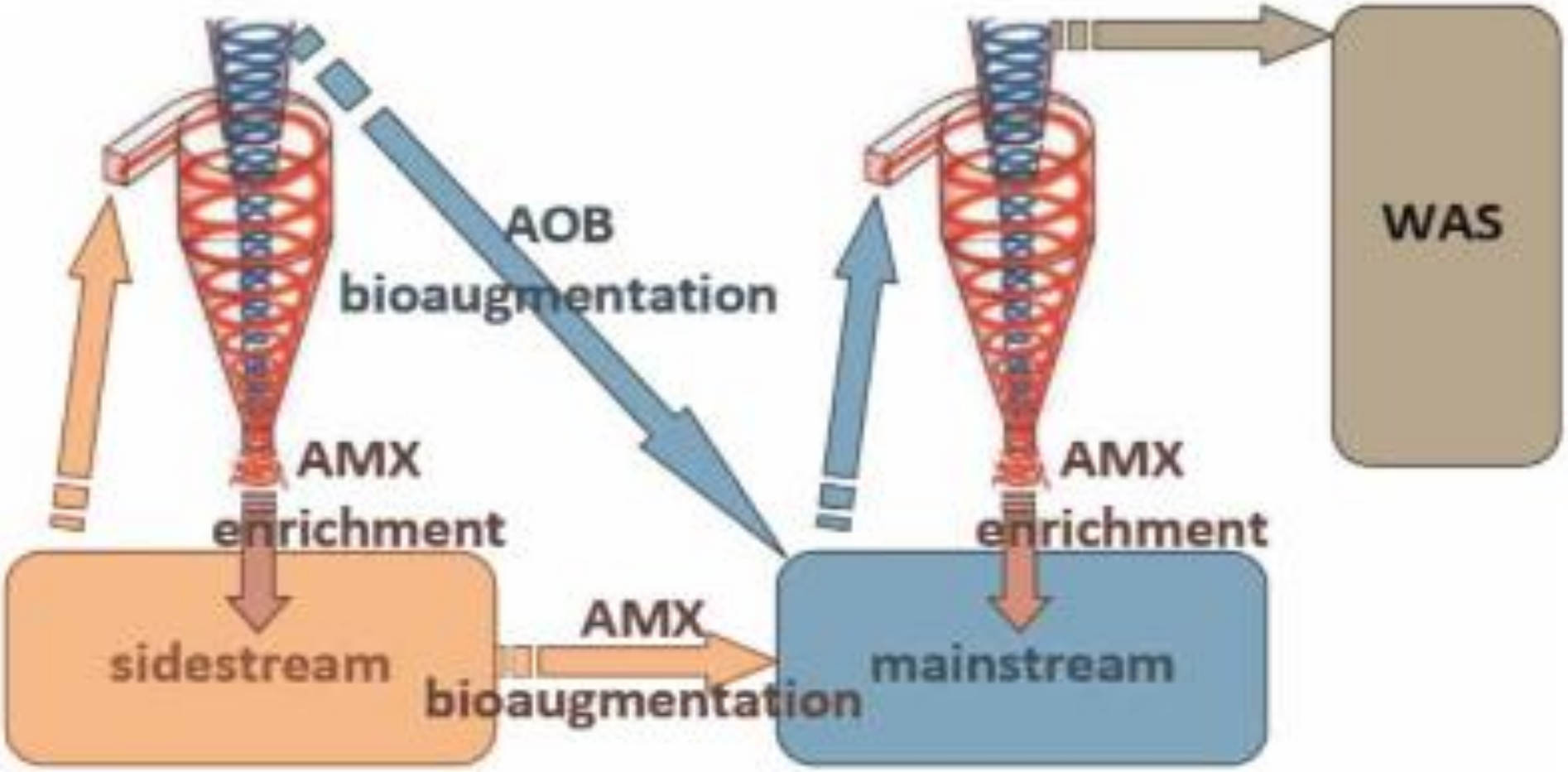


Wett et al. (2015) Going for mainstream deammonification from bench to full scale for maximized resource efficiency. Water Sci Techn 68, 283-289

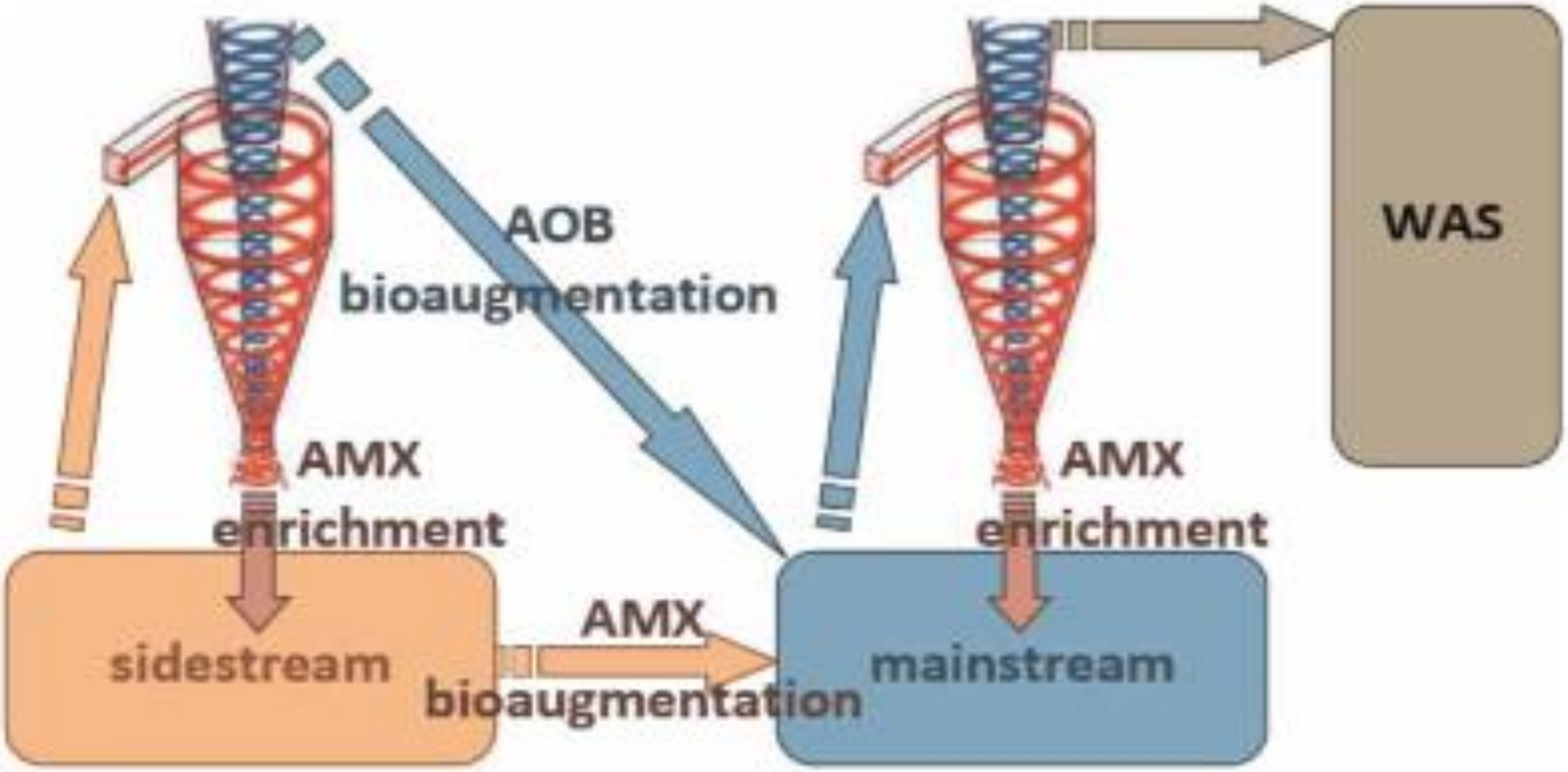
AMX (anammox)
AOB ammonia oxidising bacteria
WAS Waste overflow



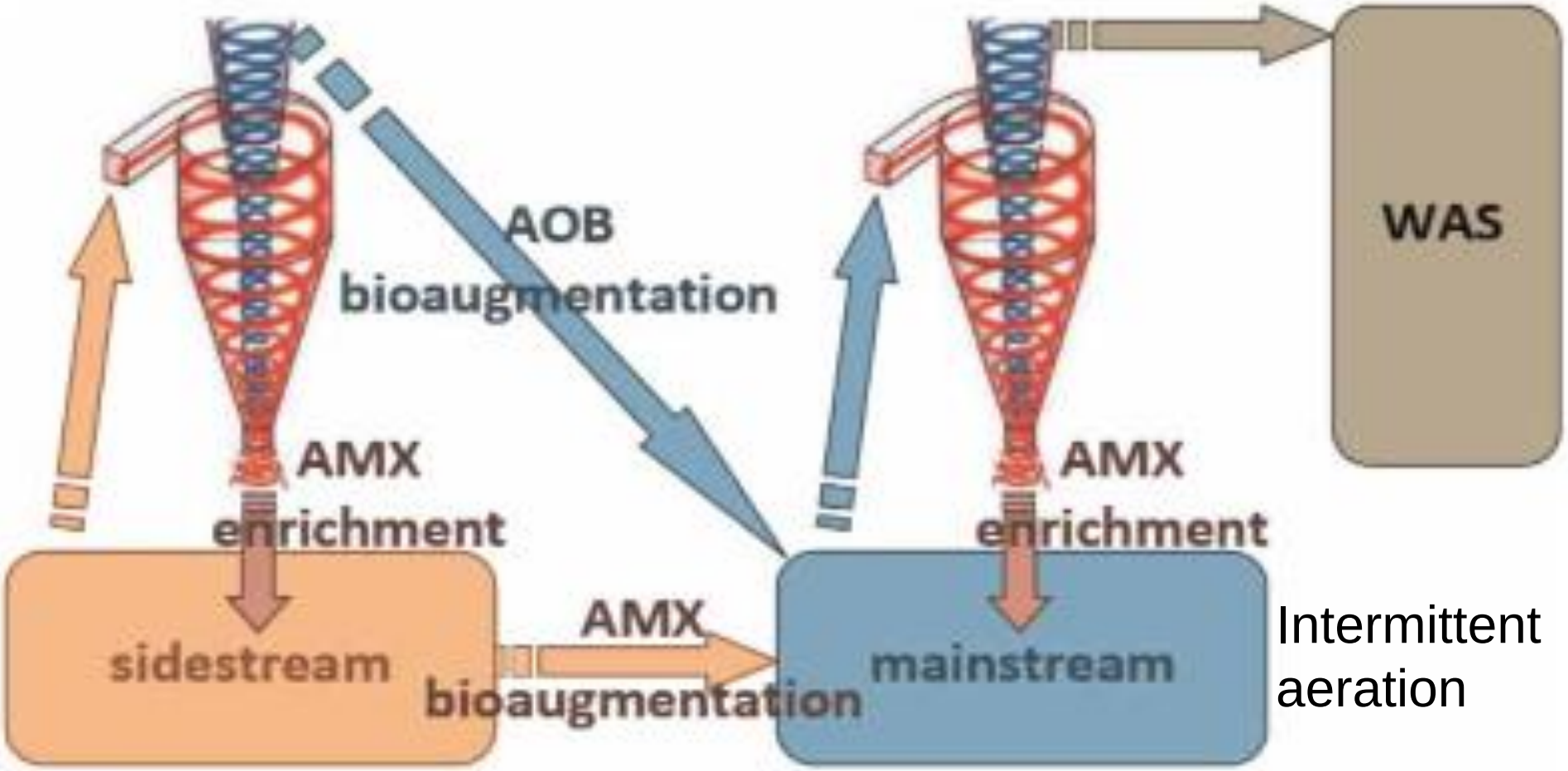
1. Bioaugmentation der AMX im Hauptstrom durch Transfer aus dem DEMON® - Nebenstrom



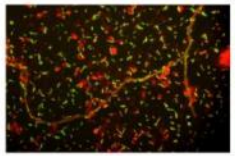
2. Bioaugmentation der Ammoniumoxidierenden Bakterien (AOB) über den Cyklon-Overflow der Nebenstrom-Reaktoren



3. Anreicherung der Anammox (AMX) – Gemeinschaft durch einen Zyklon im Hauptstrom



4. Intermittierende Belüftung im Hauptstrom reprimiert die Nitritoxidierenden Bakterien (NOB) durch vorübergehende Anoxie.

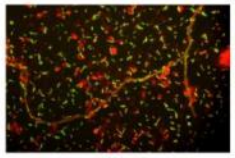


1. Bioaugmentation AMX im Haupstrom über den DEMON® Nebenstrom
2. Bioaugmentation der Ammoniumoxidierenden Bakterien (AOB)
3. Anreicherung der Anammox (AMX) durch Zyklon im Hauptstrom
4. Intermittierende Belüftung des Hauptstromes um die Nitritoxidierenden Bakterien (NOB) zu reprimieren

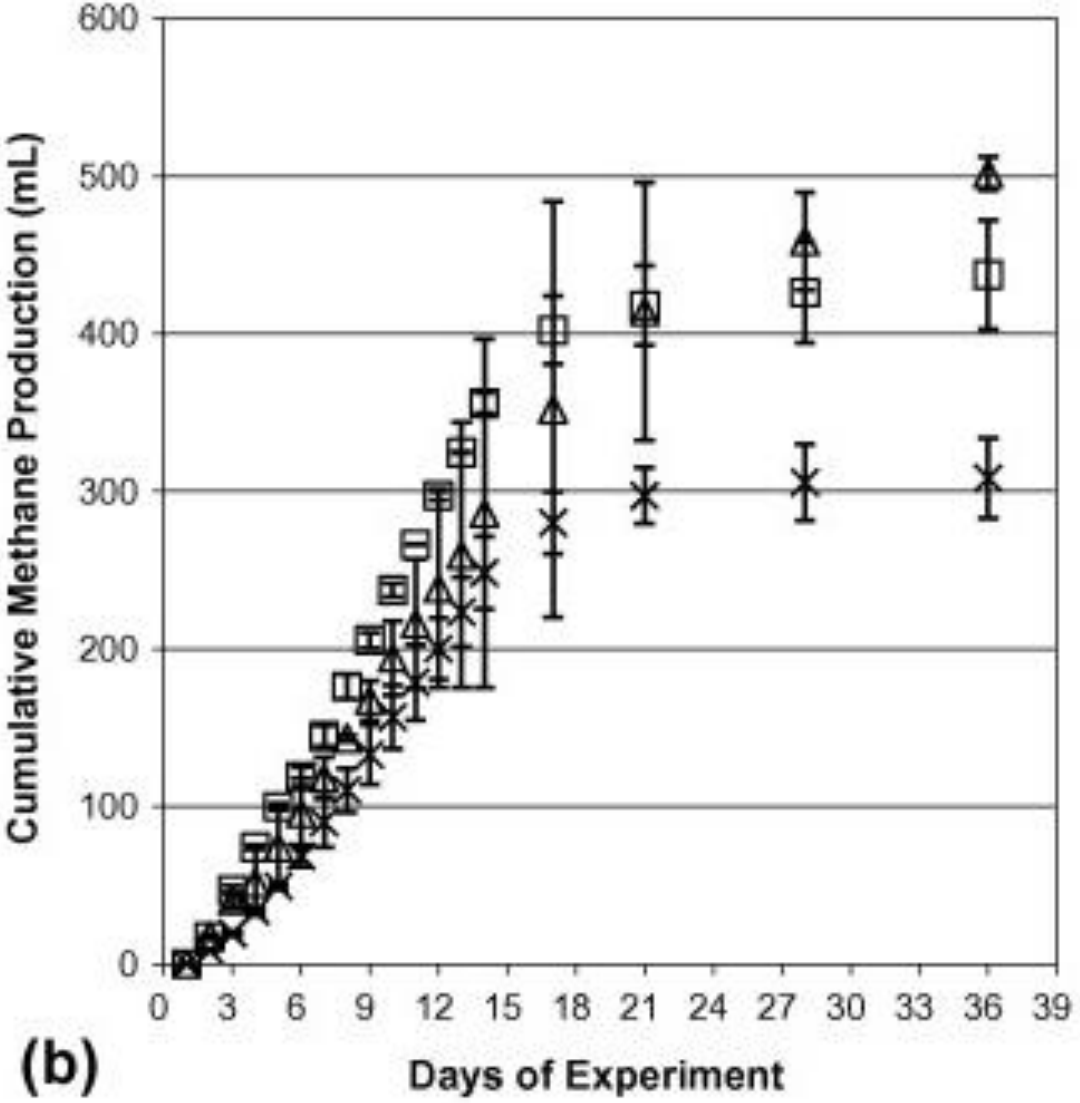
Kombination von Maßnahmen
die sich gegenseitig
unterstützen ist Voraussetzung
für den Erfolg der Maßnahme
Details: wett@araconsult.at

University of Innsbruck - Faculty of Bio

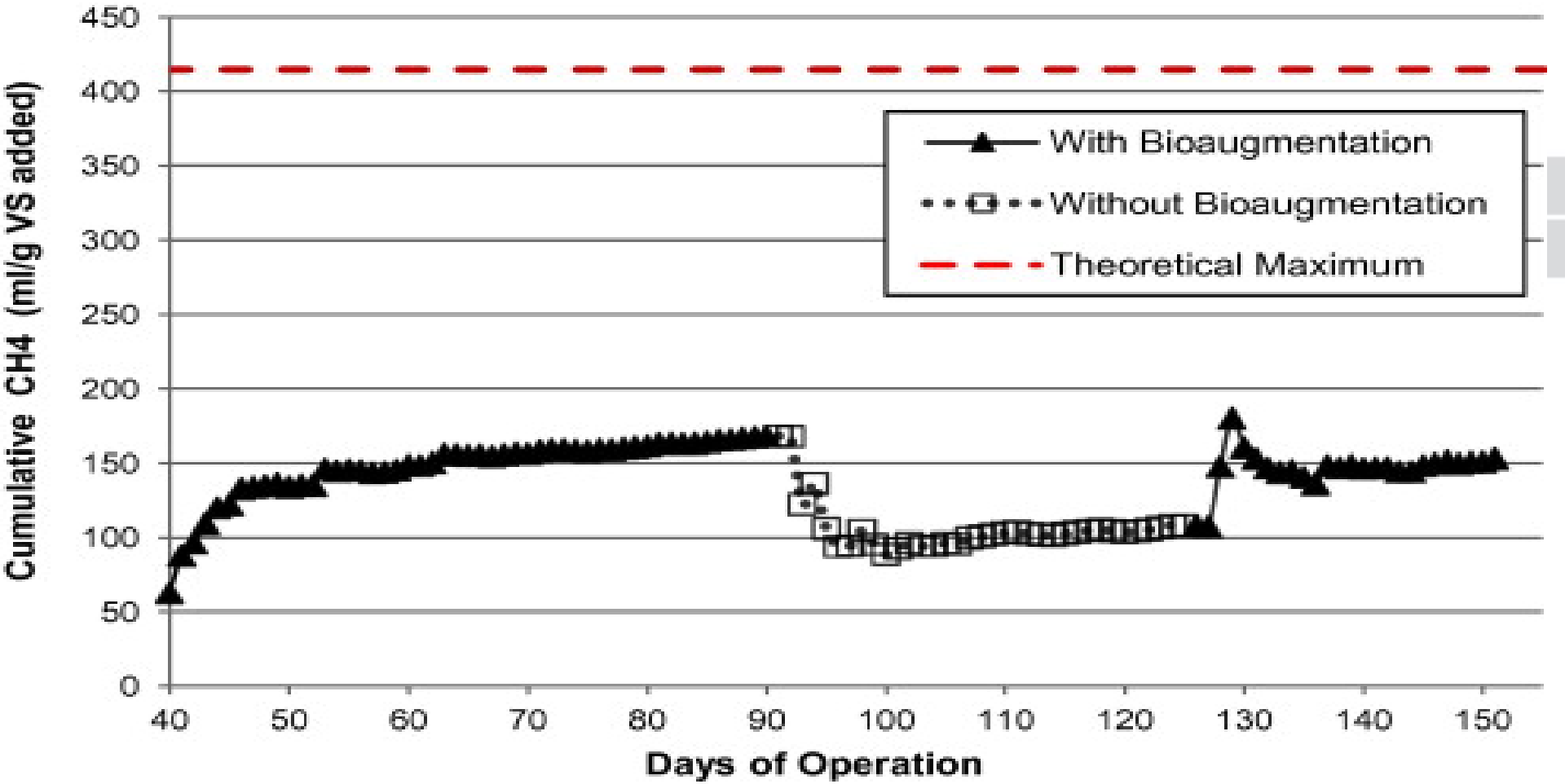
Institute of Microbiology



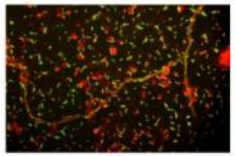
Batch-Experiment zum Vergleich der Wirkung einer proprietären cellulolytischen Mischkultur: kumulative Methanproduktion

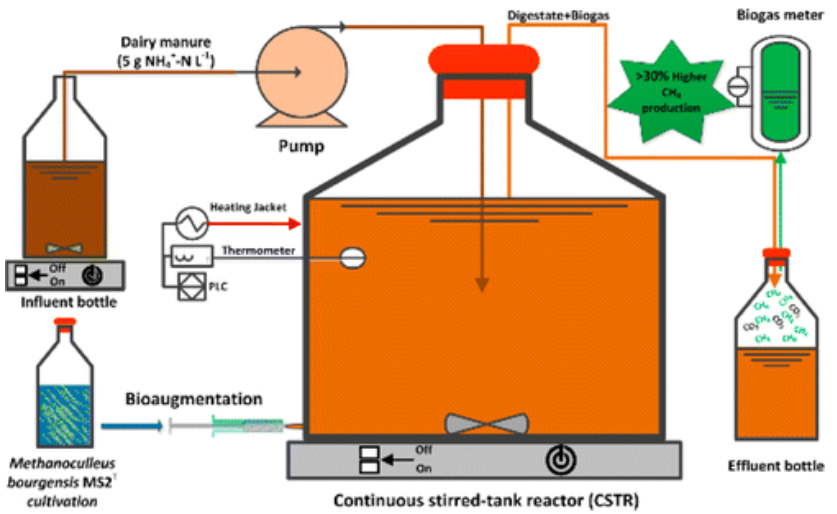


A Martin-Ryals et al. (2015) Improving anaerobic digestion of a cellulosic waste via routine bioaugmentation with cellulolytic microorganisms. Bioresource Technology, 189, 62 - 70



A proprietary **cellulolytic bioculture mixture** (predominantly dehydrated *Clostridium*) provided by Microbial Energy Systems Inc. (Bloomington, IN). In all cases of bioaugmentation, the bioculture was applied to the acid-phase of the experiment.





Fast growing hydrogenotrophic methanogen (*Methanoculleus bourgensis*) was bioaugmented in a CSTR reactor at high ammonia (5 g/L)
→ 31% increase in methane production
→ 5-fold increase in abundance of *Methanoculleus bourgensis*

Fotidis , Wang, Fiedel, Luo, Karakashev, Angelidaki (2014) Bioaugmentation as a Solution To Increase Methane Production from an Ammonia-Rich Substrate

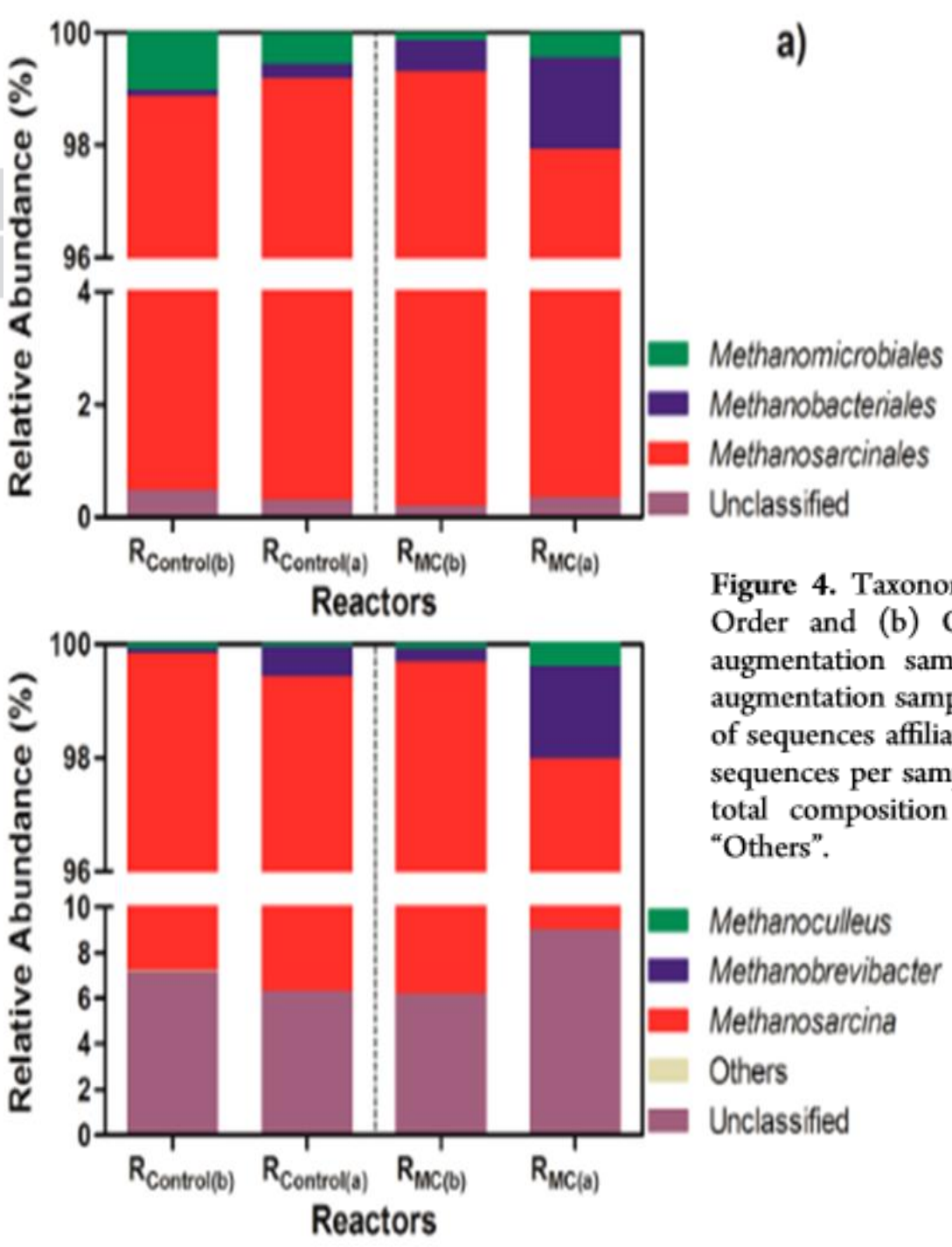
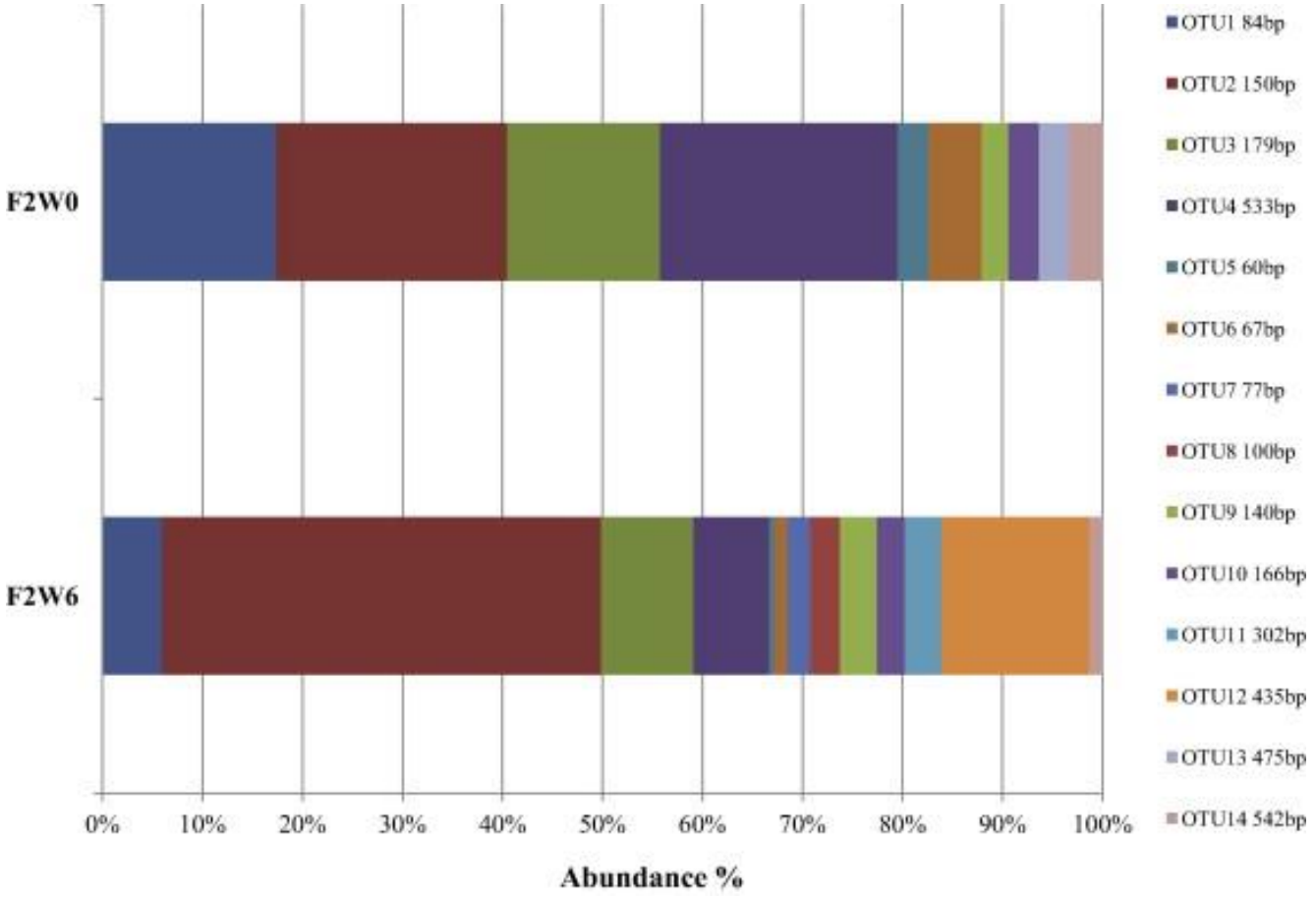
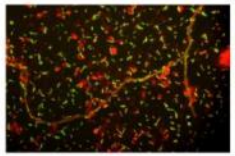


Figure 4. Taxonomic classification of the archaea communities (a) Order and (b) Genus. (b) Before bioaugmentation and abiotic augmentation samples and (a) after bioaugmentation and abiotic augmentation samples. Relative abundance was defined as the number of sequences affiliated with that taxon divided by the total number of sequences per sample. Individual genera making up less than 0.1% of total composition in all samples are summed and indicated as "Others".



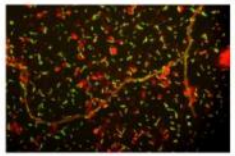
Restriction fragment distribution of the inoculated fermenter (F2) before (W0), and 6 weeks after (W6) the inoculation. The relative abundances of the various OTUs are indicated in different colors.

Ács , Bagi , Rákhely, Minárovics, Nagy, Kovács (2015) **Bioaugmentation of biogas production by a hydrogen-producing bacterium.** Bioresource Technology 186, 286 - 293



Addition of H_2 producing *Enterobacter cloacae* augmented biogas production.

- *E. cloacae* became a stable member of the biogas producing microbial community.
- Addition of *E. cloacae* significantly altered the community composition.
- Polymer degrading *Clostridiales* increased their abundance dramatically.
- A syntrophic relationship between polymer degradation and in situ H_2 generation is suggested.



Improvement of Biogas Production by Bioaugmentation
Kovács et al. 2015

Clostridium saccharolyticus with its versatile H_2-production activity augments biogas productivity from various substrates to a similar extent to *E. cloacae*.

Bioaugmentation of anaerobic reactorswith compost!!!!!

Lukas Neumann, Paul Scherer (2013) Bioaugmentation of a Biogas Fermenter Fed with Renewable Biomass by Fluorescence in Situ Hybridization

Westerholm, Levén, Schnürer (2013)

Bioaugmentation of Syntrophic Acetate-Oxidizing Culture in Biogas Reactors Exposed to Increasing Levels of Ammonia

Höhere Abundanzen von *Clostridium ultunense und Tepidanaerobacter* acetatoxydans wurden mit Bioaumentation in Zusammenhang gebracht.
Kein Einfluss konnte auf *Syntrophaceticus schinkii oder* die Methanogenen *gefunden werden.*

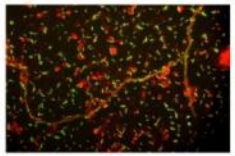
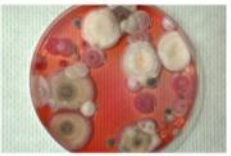
SAOBs sind wichtige Komponenten der Microbiota in AD und können stets gefunden werden, sogar in Prozessen die durch acetoklastische Methanogenese gekennzeichnet sind.
Aber: hohe Abundanzen (z.B. durch Bioaugmentation) sind nicht wichtig, vielmehr sind es die Betriebsbedingungen (z.B. hohes NH3) die eine Verschiebung zum SAO-Weg verursachen.
EVERYTHING IS EVERYWHERE

– but the environment selects

FUTURE APPLICATIONS

- Hydrogen producing bacteria
- Constructed ligno-cellulolytic cultures
- Anammox for nitrogen removal in digestate
- Anaerobic fungi and other cellulolytic MO
- Psychrophilic methanogens
- Microbial fuel cells…
- ….. Many more!

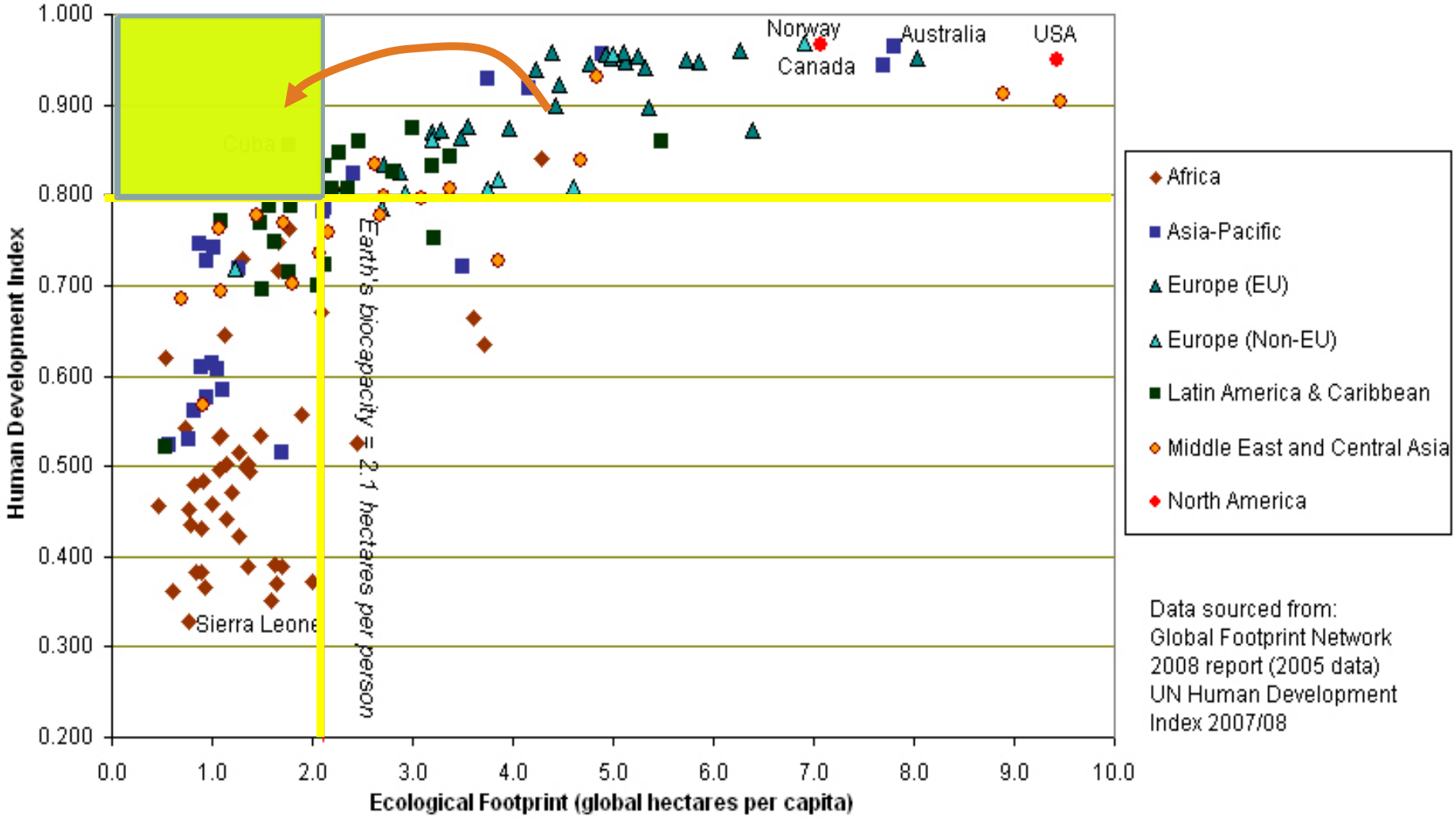
CONCLUSIONS

The superchallenges of the 21st century (®Willy Verstraete)

1. Increase of atmospheric CO_2 (greenhouse gases) → Global Warming
2. Energy crisis
3. Sustainable use of resources (water, soil, phosphorus, etc. etc.)

Human Welfare and Ecological Footprints compared



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Cooperations

Bernhard Wett, Thomas Pümpel, Ursula Peintner, Dieter Bryniok, Kate Fliegerova, Gareth Griffiths, Theodore Bouchez, Blaz Stres, Andreas Wagner, usw ….

ARAConsult
FWF
ALPs
ACIB
BioProfi MOST
Marie-Curie

Once the diversity of the microbial world is catalogued, it will make astronomy look like a pitiful science.
- Julian Davies, UBC, Canada (2003)

11.09.2011