

QuantArray®—NSZD



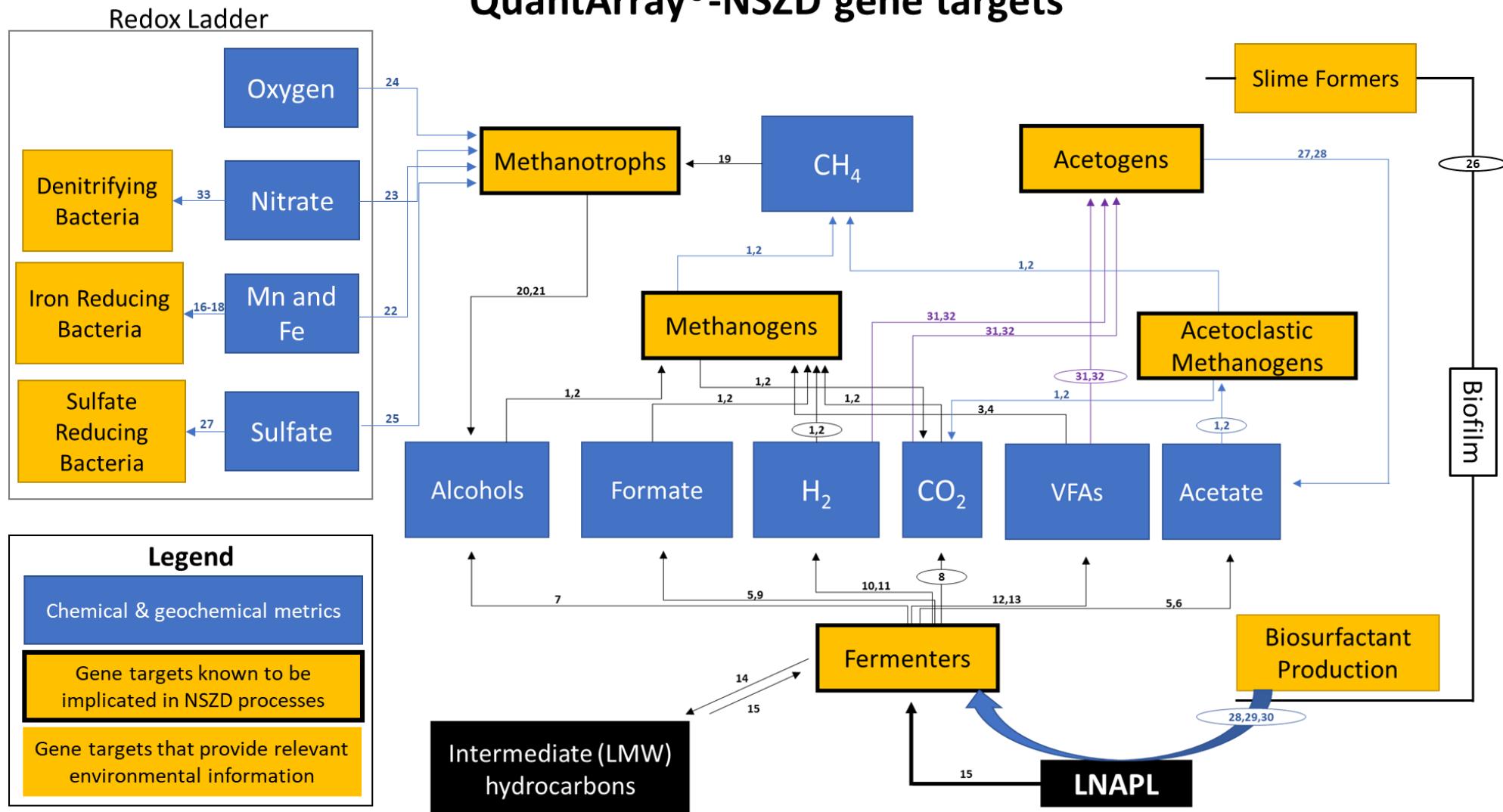
Natural source zone depletion (NSZD) is the process whereby light non-aqueous phase liquid (LNAPL) petroleum hydrocarbons are lost from the subsurface due to naturally occurring dissolution, volatilization, and biodegradation. In NSZD, biodegradation is important, although complex, and involves diverse microbial communities of not only methanogens and methanotrophs but also sulfate reducers, iron reducers, denitrifiers, fermenters, acetogens, biosurfactant producers, and slime formers.^{1, 2} To evaluate these microbial communities and the potential for biodegradation of LNAPL in the source zone, *Microbial Insights Inc.* takes your samples collected from the field, extracts RNA or DNA, and performs QuantArray®—NSZD. QuantArrays® use nanoliter fluidics and solution-phase qPCR — to quantify, in parallel, numerous genes (see below) in individually monitored reactions, in this case to accurately and comprehensively assess NSZD.

Quantification of the following microorganisms, processes, and genes:

Total microorganisms <ul style="list-style-type: none">Bacteria (EBAC)Archaea (ARC)	Sulfate Reduction <ul style="list-style-type: none">Sulfate reducing bacteria (APS)	Iron Reducing Bacteria <ul style="list-style-type: none">Bacteria (IRB)<i>Geobacter</i> (IRG)<i>Shewanella</i> (IRS)	Denitrifying Bacteria <ul style="list-style-type: none">nirKnirS
Fermenters and Acetogens <ul style="list-style-type: none">Acetogens (AGN)Fermenters (FER)	Methanogens and Methanotrophs <ul style="list-style-type: none">Methanogens (MGN)Acetoclastic methanogens (AMGN)Particulate methane monooxygenase (PMMO)Soluble methane monooxygenase (SMMO)	Biosurfactant Production <ul style="list-style-type: none">Glycolipid (SurG)Liposaccharide (SurL)Lipopptide (SurP)Trehalose (SurT)	Slime Production <ul style="list-style-type: none"><i>Burkholderia cepacia</i> exopolysaccharide (BCE)<i>Deinococcus</i> spp. (DCS)<i>Meiothermus</i> spp. (MTS)

- Johnson P, Lundegard P, Liu Z: Source zone natural attenuation at petroleum hydrocarbon spill sites—I: Site-specific assessment approach. *Groundwater Monitoring & Remediation*. 2006;26:82-92.
- Pannekens M, Kroll L, Muller H, Mbow FT, Meckenstock RU: Oil reservoirs, an exceptional habitat for microorganisms. *N Biotechnol*. 2019;49:1-9.

QuantArray®-NSZD gene targets



QuantArray®-NSZD Target Overview

Methanogens:

Alcohols/Methanol

1. Enzman, F., F. Mayer, M. Rother, and D. Holtmann. 2018. Methanogens: biochemical background and biotechnological applications. *AMB Express* 8(1).
2. Buan, N. 2018. Methanogens: pushing the boundaries of biology. *Emerging Topics in Life Sciences*. 2: 629-646.

Formate

1. Enzman, F., F. Mayer, M. Rother, and D. Holtmann. 2018. Methanogens: biochemical background and biotechnological applications. *AMB Express* 8(1).
2. Buan, N. 2018. Methanogens: pushing the boundaries of biology. *Emerging Topics in Life Sciences*. 2: 629-646.

H₂/CO₂

1. Enzman, F., F. Mayer, M. Rother, and D. Holtmann. 2018. Methanogens: biochemical background and biotechnological applications. *AMB Express* 8(1).
2. Buan, N. 2018. Methanogens: pushing the boundaries of biology. *Emerging Topics in Life Sciences*. 2: 629-646.

Acetate

1. Enzman, F., F. Mayer, M. Rother, and D. Holtmann. 2018. Methanogens: biochemical background and biotechnological applications. *AMB Express* 8(1).
2. Buan, N. 2018. Methanogens: pushing the boundaries of biology. *Emerging Topics in Life Sciences*. 2: 629-646.

VFAs

3. Yamaguchi, M., K. Minami, Y. Tanimoto, and K. Okamura. 1989. Effects of volatile fatty acids on methanogenesis of methanol and of pregrowth with methanol on acetate utilization by methanogens. *Journal of Fermentation and Bioengineering* 68: 428-432.
4. Wang, Y., Y. Zhang, J. Wang, and L. Meng. 2009. Effects of volatile fatty acid concentrations on methane yield and methanogenic bacteria. *Biomass and Bioenergy* 33: 848-853.

Fermenters

Acetate Production

5. Cselovszky, J., G. Wolf, and W. Hammes. 1992. Production of formate, acetate, and succinate by anaerobic fermentation of *Lactobacillus pentosus* in the presence of citrate. *Applied Microbiology and Biotechnology* 37: 94-97.
6. Zhang, B., C. Lingga, C. Bowman, and T. Hackmann. 2021. A new pathway for forming acetate and synthesizing ATP during fermentation in bacteria. *Applied and Environmental Microbiology* 87.

Alcohol Production:

7. NG, T., A. Ben-Bassat, and J. Zeikus. 1981. Ethanol production by thermophilic bacteria: fermentation of cellulosic substrates by cocultures of *Clostridium thermocellum* and *Clostridium thermohydrosulfuricum*. *Applied and Environmental Microbiology* 41(6): 1337-1343.

CO₂ Production

8. Gruca-Rokosz, R., and P. Koszelnik. 2018. Production pathways for CH₄ and CO₂ in sediments of two freshwater ecosystems in south-eastern Poland. *PLoS ONE* 13(6).

Formate Production

5. Cselovszky, J., G. Wolf, and W. Hammes. 1992. Production of formate, acetate, and succinate by anaerobic fermentation of *Lactobacillus pentosus* in the presence of citrate. *Applied Microbiology and Biotechnology* 37: 94-97.

9. Oswald, F., I. Stoll, M. Zwic, S. Herbif, J. Sauer, N. Boukis, and A. Neumann. 2018. Formic acid formation by *Clostridium ljungdahlii* at elevated pressures of carbon dioxide and hydrogen. *Frontiers in Bioengineering and Biotechnology* 6.

Hydrogen Production

10. Yesanew, M., F. Paillet, C. Barrau, I. Frunzo, P. Lens, G. Esposito, R. Escudie, and E. Trably. 2018. Co-production of Hydrogen and Methane from the organic fraction of municipal solid waste in a pilot scale dark fermenter and methanogenic biofilm reactor. *Frontiers in Environmental Science* 6(41).

11. Su, X., W. Zhao, and D. Xia. 2018. The diversity of hydrogen-producing bacteria and methanogens within an in situ coal seam. *Biotechnology and Biofuels* 11 (245).

VFA production

12. Strain, B., M. Sobarzo, G. Daneri, H. Gonzalez, G. Testa, L. Farias, A. Schwarz, N. Perez, and S. Pantoja-Gutierrez. 2020. Fermentation and anaerobic oxidation of organic carbon in the oxygen minimum zone of the upwelling ecosystem off Concepcion, in Central Chile. *Frontiers in Marine Science* 7(533).

13. Pandey, A., S. Pilli, P. Bhunia, R. Tyagi, R. Surampalli, T. Zhang, S. Kim, and A. Pandey. Dark fermentation: production and utilization of volatile fatty acid from different wastes- a review. *Chemosphere* 288.

Intermediate PH production

14. Hou, N., N. Zhang, T. Jia, Y. Sun, Y. Dai, Q. Wang, D. Li, Z. Luo, and C. Li. 2018. Biodegradation of phenanthrene by biodemulsifier-producing strain Achromobacter sp. LH-1 and the study on its metabolisms and fermentation kinetics. *Ecotoxicology and Environmental Safety* 163: 205-214.

LNAPL fermentation

15. Lari, K.S., G.B. Davis, J.L. Rayner, T.P. Bastow, and G.J. Puzon. 2019. Natural source zone depletion of LNAPL: A critical review supporting modelling approaches. *Water Research* 157: 630-646.

Iron Reducing Bacteria

16. Esther, J., L. Sukla, N. Pradhan, and S. Panda. 2015. Fe(III) reduction strategies of dissimilatory iron reducing bacteria. *Korean Journal of Chemical Engineering*.

17. Kostka, J.E., G.W. Luther III, and K.H. Nealson. 1995. Chemical and biological reduction of Mn(III)-pyrophosphate complexes: potential importance of dissolved Mn(III) as an environmental oxidant. *Geochimica et Cosmochimica* 59(5): 885-894.

18. Szeinbaum, N., H. Lin, J.A. Brandes, M. Taillefert, J.B. Glass, and T.J. DiChristina. 2017. Microbial manganese(III) reduction fuelled by anaerobic acetate oxidation. *Environmental Microbiology* 19(9): 3475-3486.

Methanotrophs

19. Guerrero-Cruz, S., A. Vaksmaa, M. Horn, H. Niemann, M. Pijuan, and A. Ho. 2021. Methanotrophs: discoveries environmental relevance, and a perspective on current and future applications. *Frontiers in Microbiology* 12.

Alcohol Production

20. Oh, S., I. Hwang, O. Lee, W. Won, and E. Lee. 2019. Development and optimization of the biological conversion of ethane to ethanol using whole-cell methanotrophs possessing methane monooxygenases. *Molecules* 24(591).

21. Xin, J., J. Cui, J. Niu, S. Hua, C. Xia, S. Li, and L. Zhu. 2004. Production of methanol from methane by methanotrophic bacteria. *Biocatalysis ad Biotransformation* 22: 225-229.

Iron and Manganese

22. Ettwig, K., B. Zhu, D. Speth, J. Keltjens, M. Jetten, and B. Kartal. 2016. Archaea catalyze iron-dependent anaerobic oxidation of methane. *PNAS* 113: 12792-12796.

Nitrate

23. Ettwig, K., S. Shima, K. van de Pas-Schoonen, J. Kahnt, M. Medema, H. op den Camp, M. Jetten, and M. Strous. 2008. Denitrifying bacteria anaerobically oxidize methane in the absence of archaea. *Environmental Microbiology* 10: 3164-3173.

Redox Oxygen

24. Ettwig, K., D. Speth, J. Reimann, M. Wu, M. Jetten, and J. Keltjens. 2012. Bacterial oxygen production in the dark. *Frontiers in Microbiology* 3.

Sulfate

25. Boetius, A., K. Ravenschlag, C. Schubert, D. Rickert, F. Widdel, A. Gieseke, R. Ama, B. Jorgensen, U. Witte, and O. Pfannkuche. 2001. A marine microbial consortium apparently mediating anaerobic oxidation of methane. *Letters to Nature*. 407: 623-626.

Slime Formers

26. Pannekens, M., L. Kroll, H. Muller, F. Mbow, R. Meckenstock. 2019. Oil reservoirs, an exception habitat for microorganisms. *New Biotechnology* 49: 1-9.

Sulfate reducing bacteria

27. Muyzer, G. and A. Stams. 2008. The ecology and biotechnology of sulphate reducing bacteria. *Nature Reviews* 6: 441-454.

Biosurfactants

28. Wang, X., T. Cai, W. Wen, J. Ai, J. Ai, Z. Zhang, L. Zhu, and S. George. 2020. Surfactin for enhanced removal of aromatic hydrocarbons during biodegradation of crude oil. *Fuel* 267.

29. Bognolo, G. Biosurfactants as emulsifying agents for hydrocarbons. 1999. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 152: 41-52.

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Acetogens

31. Karekar, S., R. Stefanini, and B. Ahring. 2022. Homo-acetogens: their metabolism and competitive relationship with hydrogenotrophic methanogens. *Microorganisms* 10(397).

32. Muller, V. 2003. Energy conservation in acetogenic bacteria. *Applied and Environmental Microbiology* 69: 6345-6353.

Denitrifying Bacteria

33. Houlton, B. and E. Bai. 2009. Imprint of denitrifying bacteria on the global terrestrial biosphere. *PNAS* 106: 21713-21732.