Microbiology of synthesis gas fermentation for biofuel production

Anne M Henstra[1], Jan Sipma[2], Arjen Rinzema[3] and Alfons JM Stams[1]

A significant portion of biomass sources like straw and wood is poorly degradable and cannot be converted to biofuels by microorganisms. The gasification of this waste material to produce synthesis gas (or syngas) could offer a solution to this problem, as microorganisms that convert CO and H_2 (the essential components of syngas) to multicarbon compounds are available. These are predominantly mesophilic microorganisms that produce short-chain fatty acids and alcohols from CO and H_2. Additionally, hydrogen can be produced by carboxydotrophic hydrogenogenic bacteria that convert CO and H_2O to H_2 and CO_2. The production of ethanol through syngas fermentation is already available as a commercial process. The use of thermophilic microorganisms for these processes could offer some advantages; however, to date, few thermophiles are known that grow well on syngas and produce organic compounds. The identification of new isolates that would broaden the product range of syngas fermentations is desirable. Metabolic engineering could be employed to broaden the variety of available products, although genetic tools for such engineering are currently unavailable. Nevertheless, syngas fermenting microorganisms possess advantageous characteristics for biofuel production and hold potential for future engineering efforts.

Addresses
[1] Laboratory of Microbiology, Wageningen University, H. v. Suchtelenweg 4, 6703 CT, Wageningen, The Netherlands
[2] Chemical and Environmental Engineering Laboratory (LEQUiA), University of Girona, Campus Montilivi s/n, E-17071 Girona, Spain
[3] Food & Bioprocess Engineering, Wageningen University, PO Box 8129, 6700 EV, Wageningen, The Netherlands

Corresponding author: Henstra, Anne M (anne-meint.henstra@wur.nl)

Current Opinion in Biotechnology 2007, **18**:200–206

This review comes from a themed issue on
Energy biotechnology
Edited by Lars Angenent

Available online 30th March 2007

0958-1669/$ – see front matter

DOI 10.1016/j.copbio.2007.03.008

Introduction

The use of oil and other fossil resources as transportation fuels and commodity chemicals is deeply engrained in today's society, but use of these resources is unsustainable. The unsustainable nature of fossil fuels stems from their finite reserves and their negative environmental impact: combustion of fuels releases carbon dioxide and various pollutants, such as sulfur and nitrogen oxides. Thus, there is a need for alternative processes to produce energy and chemicals [1•,2]. The transition towards a sustainable energy supply will take considerable time. In the meantime, short-term solutions will aim to lessen the environmental impact of fossil fuels [3].

Cleaner fuels are obtained through improved refining technologies and through the addition of synthetic fuels or ethanol. These latter options have interesting future potential as they can be derived from biomass. Bioethanol is predominantly produced through the fermentation of easily degradable carbohydrate substrates, such as corn starch and sugar cane. Alternatively, fermentable sugars can be obtained through the acid or enzymatic pretreatment of insoluble cellulosic biomass [1•,2]. However, most biomass sources like straw and wood contain a large proportion of material that cannot be converted to ethanol by microorganisms. An alternative might be to gasify organic biomass and to use the produced synthesis gas (or syngas) as a feed stock for the synthesis of ethanol and other valuable compounds. Syngas, formed by the gasification or reforming of coal, natural gas or biomass, is a key intermediate in the production of synthetic fuels [4]. As syngas can be produced from both fossil fuels and renewable resources, it also enables a gradual transition to more sustainable energy and chemical production. Carbon monoxide and molecular hydrogen are the essential components of syngas and are used as building blocks in processes like Fischer-Tropsch synthesis to form linear alkanes [4]. Pure hydrogen is produced from syngas through the water gas shift reaction (WGS) according to the reaction: $CO + H_2O \rightarrow CO_2 + H_2$ [5].

The CO and H_2 present in syngas are substrates for microbial metabolism, which can be exploited for the synthesis of various interesting products. It is expected that syngas fermentation will play a role in the conversion of biomass, wastes and residues that form poor substrates for direct fermentation [1•,2,6,7]. As gasification results in gas with a high temperature, thermophilic microbial processes might be most applicable for the biotechnological production of chemicals from syngas. Here we review syngas fermentation with a focus on microbiological aspects and indicate areas where advances can be made.

Syngas fermentation

The production of fuels and chemicals through syngas fermentation offers several advantages over metal catalytic conversion. The higher specificity of the biocatalyst, lower energy costs, greater resistance to catalyst poisoning, and independence of a fixed H_2:CO ratio are generally

mentioned [8,9]. In the past two decades, new isolates and some known anaerobic microorganisms were shown capable of growth with CO and H_2 as substrates (Table 1). Although most strains showed the formation of acetate, formate and butyrate, ethanol and butanol were also reported as products. Additionally, several purple non-sulfur bacteria were isolated that are able to convert CO to H_2 in a process similar to the WGS reaction (Table 1).

The fermentation of syngas to ethanol by *Clostridium ljungdahlii* was developed into a commercial process that combines biomass gasification, syngas fermentation and distillation of ethanol from the reactor effluent. Syngas is cooled before it can be introduced into the bioreactor and is coupled to heat recovery (BRI energy; URL: http://www.brienergy.com/). Processes that use the biological WGS reaction to produce hydrogen from syngas are still at labscale. The lower temperatures of the biological gas-shift favour CO conversion to H_2 with lower CO concentrations, compared with WGS [5]. In experiments with *Carboxydothermus hydrogenoformans*, CO thresholds below 2 ppm could be obtained if CO_2 was removed from the gas phase of batch cultures. Without CO_2 removal, 117 ppm CO remained from an original gas phase of 100% CO, whereas chemical technology generally leaves 1000 ppm of CO (AM Henstra, PhD thesis, Wageningen University 2006). Low CO concentrations are required for application of the produced H_2 gas in CO-sensitive low temperature fuel cells [10].

Generally, gas/liquid mass transfer limits conversion rates in bioprocesses that use sparingly soluble gases [9]. High gas and liquid flow rates, large specific gas–liquid interfacial areas, and increased gas solubility (through the use of increased pressure or solvents), stimulate gas/liquid mass transfer rates. Continuous stirred tank reactors

Table 1

Anaerobic carboxydotrophic microorganisms.

Species	T_{opt} (°C)	pH_{opt}	t_d (h)	Products	Reference
Mesophilic bacteria					
Clostridium autoethanogenum	37	5.8–6.0	nr	Acetate, ethanol	[34]
Clostridium ljungdahlii	37	6	3.8	Acetate, ethanol	[35]
Clostridium carboxidivorans	38	6.2	6.25	Acetate, ethanol, butyrate, butanol	[36]
Oxobacter pfennigii	36–38	7.3	13.9	Acetate, n-butyrate	[37]
Peptostreptococcus productus	37	7	1.5	Acetate	[38]
Acetobacterium woodii	30	6.8	13	Acetate	[39]
Eubacterium limosum	38–39	7.0–7.2	7	Acetate	[39,40]
Butyribacterium methylotrophicum	37	6	12–20	Acetate, ethanol, butyrate, butanol	[41–43]
Rubrivivax gelatinosus	34	6.7–6.9	6.7	H_2	[44,45]
Rhodopseudomonas palustris P4	30	nr	23	H_2	[46]
Rhodospirillum rubrum	30	6.8	8.4	H_2	[47]
Citrobacter sp Y19	30–40	5.5–7.5	8.3	H_2	[48,49]
Mesophilic archaea					
Methanosarcina barkeri	37	7.4	65	CH_4	[50]
Methanosarcina acetivorans strain C2A	37	7	24	Acetate, formate, CH_4	[51]
Thermophilic bacteria					
Moorella thermoacetica	55	6.5–6.8	10	Acetate	[17]
Moorella thermoautotrophica	58	6.1	7	Acetate	[19]
Moorella strain AMP	60–65	6.9	nr	H_2	[a]
Carboxydothermus hydrogenoformans	70–72	6.8–7.0	2	H_2	[31]
Carboxydibrachium pacificus	70	6.8–7.1	7.1	H_2	[52]
Carboxydocella sporoproducens	60	6.8	1	H_2	[15•]
Carboxydocella thermoautotrophica	58	7	1.1	H_2	[53]
Thermincola carboxydiphila	55	8	1.3	H_2	[54]
Thermincola ferriacetica	57–60	7.0–7.2	nr		[55]
Thermolithobacter carboxydivorans[b]	70	7	8.3	H_2	[56,57]
Thermosinus carboxydivorans	60	6.8–7.0	1.2	H_2	[58]
Desulfotomaculum kuznetsovii	60	7	nr	Acetate, H_2S	[59]
Desulfotomaculum thermobenzoicum subsp. *thermosyntrophicum*	55	7	nr	Acetate, H_2S	[59]
Desulfotomaculum carboxydivorans	55	7	1.7	H_2, H_2S	[16•]
Thermophilic archaea					
Methanothermobacter thermoautotrophicus	65	7.4	140	CH_4	[60]
Thermococcus strain AM4	82	6.8	nr	H_2	[61]
Archaeoglobus fulgidus	83	6.4	nr	Acetate, formate, H_2S	[22•]

[a](B Jiang, B Jiang, PhD thesis, Wageningen University, 2006); [b]*T. carboxydivorans* was previously known as *Carboxydothermus restrictus* R1. nr, not reported.

(CSTR) offer high gas/liquid mass transfer coefficients (K_La) at high impeller speeds, thus high power consumption [8]. High impeller speeds effectively break up large bubbles into smaller bubbles with more beneficial surface/volume ratios. Small bubbles additionally have lower rise velocities, thus longer liquid contact time. In microbubble dispersion, extremely small, surfactant-stabilised bubbles are created in a high shear zone, providing a more energy efficient method to increase K_La values [8]. In a study that addressed CO conversion in three types of reactors, it was found that a biotrickling filter gave higher efficiencies than CSTR and bubble column reactors. This was attributed to operational conditions that approach plug flow [9]. Furthermore, in biotrickling filters the K_La is relatively independent of the gas flow rate for sparingly soluble gasses [8]. Additionally, a low pressure drop is associated with trickle-bed reactors, ensuring relatively low power consumption. Novel bioreactor types designed to handle gases might be of interest for syngas fermentations. Monolith biofilm reactors resemble trickle-bed reactors in that the biomass is present as a biofilm attached to a carrier material and gas is led along the biofilm surface. In monoliths the pressure drop is lower than in randomly packed beds, owing to the large open frontal area [11]. In a membrane biofilm reactor (MBfR) a biofilm is directly attached to a membrane through which gases used by the biomass diffuse [12]. Hollow fibre MBfRs have been proposed as technologically and economically feasible for the hydrogen-based removal of oxidized contaminants from drinking water [13]. Elevated pressures in syngas fermentations are desired; these allow higher mass transfer rates and reduce the gas volume, thus potentially reducing the reactor size. Pressure-tolerant microorganisms that resist gas pressures of 40–50 Mpa exist [14]. Under mass transfer limitations, the CO concentrations in the liquid phase will be close to zero [9]. However, if mass transfer is improved or in the case of disturbances, the biomass concentration can become limiting. CO concentrations will then rise to equilibrium, possibly affecting CO-sensitive microorganisms and resulting in an unstable process [8].

Predominantly, mesophilic organisms have been shown to form organic compounds from syngas. So far, few attempts have been made to isolate thermophilic microorganisms that can produce organic compounds from syngas. Growth by the thermophiles at high temperatures could be advantageous, as less cooling of the syngas is required before it is introduced into the bioreactor. Additionally, higher temperatures can lead to higher conversion rates and benefit separation of the product by distillation (e.g. of ethanol). However, higher temperatures do have a negative impact on the solubility of CO and H_2.

Carboxydotrophic thermophiles

The number of Gram-positive thermophiles known to be capable of growth on CO as substrate has increased considerably in the past decade. For example, *Carboxydocella sporoproducens* was isolated from a thermal spring of the volcanic Karymskoe Lake [15•], while *Desulfotomaculum carboxydivorans* was isolated from anaerobic bioreactor sludge of a wastewater treatment plant [16•]. Two thermophilic microorganisms capable of growth on CO have been known for a long time: the homoacetogens *Moorella thermoacetica* (previously *Clostridium thermoaceticum*) and *Moorella thermoautotrophica* (previously *Clostridium thermoautotrophicum*), which both convert CO to acetate. These species have optimum growth temperatures of 55 °C and 58 °C, respectively, and doubling times of 10 h and 7 h with CO [17–19]. *M. thermoautotrophica* proved sensitive to CO, but this could be partially relieved by increasing the CO_2 partial pressures [19]. As neither homoacetogen was isolated using CO as a substrate, they might not perform optimally with CO.

The recently isolated thermophiles are predominantly carboxydotrophic hydrogenogens that grow chemolithoautotrophically through the conversion of CO and H_2O to H_2 and CO_2. Temperature optima for growth range from 55 °C to 80 °C and most specific growth rates reported are between 1 and 2 h^{-1} (Table 1). Some are obligate chemolithoautotrophs, while others might also grow organotrophically or reduce various electron acceptors with CO or H_2 as electron donor. None of the carboxydotrophic hydrogenogenic isolates forms organic compounds with CO (Table 1).

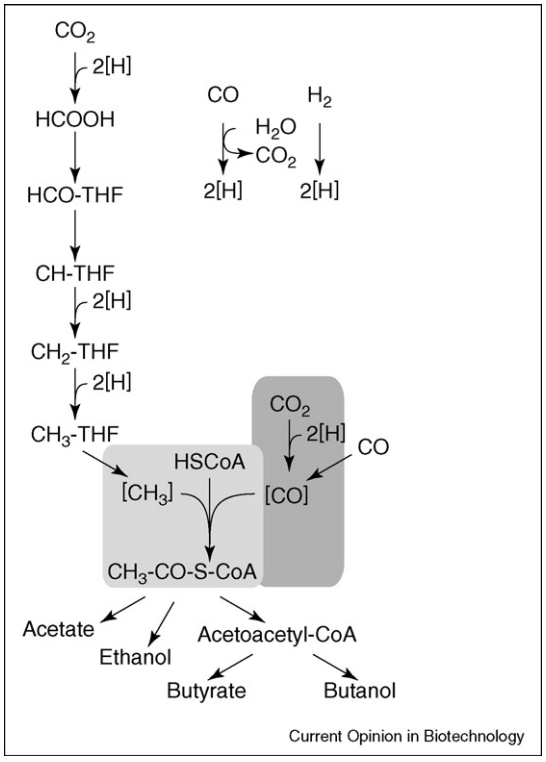
Whole genome sequences indicate that other microorganisms may grow well with CO as substrate. The genomes of *Thermoanaerobacter tengcongensis* and *Archaeoglobus fulgidus* encode CO dehydrogenases that are known to be important for growth with CO by carboxydotrophic anaerobes [20,21]. The hyperthermophilic sulfate-reducing archaeon *Archaeoglobus fulgidus* was recently adapted to growth with CO. Generally *A. fulgidus* is cultivated at 80 °C with lactate and sulfate as growth substrates. After adaptation by transferring cells to lactate-free serum bottles containing a CO gasphase, *A. fulgidus* grew with CO in the presence and absence of sulfate and produced acetate and transiently formate [22•].

If conditions are chosen properly, it seems likely that it will be possible to isolate thermophilic microorganisms that grow well with CO and which produce organic compounds of greater interest than acetate (e.g. ethanol and butanol). However, thus far, few attempts have been made in this direction.

The acetyl-CoA pathway and CO dehydrogenase

For the production of acetate, ethanol, butyrate and butanol, syngas-fermenting microorganisms depend on the acetyl-CoA pathway (Figure 1). The acetyl-CoA pathway is present in bacteria as well as Achaea, albeit with

Figure 1



Schematic representation of the reductive acetyl-CoA pathway of bacteria and the pathway for the formation of organic acids and alcohols from acetyl-CoA. Oxidation of H_2 to $2H^+$ or of CO with H_2O to CO_2 and $2H^+$ provides reducing equivalents for the reduction of CO_2 to formate (HCOOH), of methylene-tetrahydrofolate (CH-THF) to methenyl-tetrahydrofolate (CH_2-THF), of CH_2-THF to methyl-tetrahydrofolate (CH_3-THF), and of CO_2 to CO. Acetyl-CoA synthase/CO dehydrogenase catalyses the formation of acetyl-CoA from a bound methyl group, a bound CO group and coenzyme A (CoA) (grey box). Only reductive steps of the acetyl-CoA pathway are indicated.

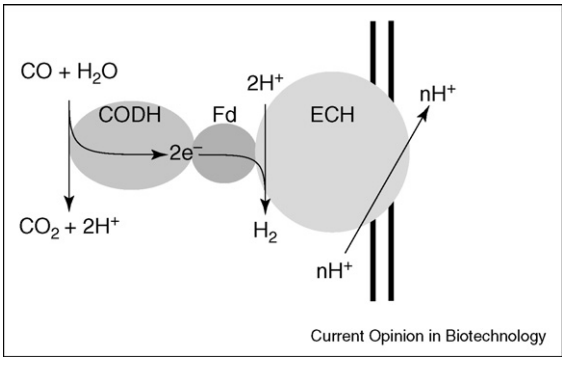
slight differences. It is likely that the acetyl-CoA pathway is restricted to anaerobes. In the non-cyclic pathway coenzyme A (CoA), a carbonyl and a methyl group are joined by an acetyl-CoA synthase/carbon monoxide dehydrogenase complex (ACS/CODH) to form acetyl-CoA [23,24]. The bifunctional CO dehydrogenase of the complex is responsible for the reduction of CO_2 to form CO, which serves as the carbonyl group. In cases where CO is readily available the CO dehydrogenase is not necessarily needed, as demonstrated for *Carboxydothermus hydrogenoformans* [25,26]. The methyl group is obtained by the reduction of CO_2 in several successive steps with formyl, methenyl, methylene and methyl intermediates bound to a pterin cofactor. In bacteria, CO_2 is first reduced to formate which is then activated at the expense of ATP to form a formyl bound to the pterin tetrahydrofolate (Figure 1). In Achaea the CO_2 is reduced to a methanofuran-bound formyl, which is subsequently transferred to tetrahydromethanopterin [27]. The formation of acetyl-CoA from H_2/CO_2 has a negative energy balance. Acetate is formed from acetyl-CoA to recover metabolic energy that is invested earlier in the acetyl-CoA pathway. Further reduction of acetate yields ethanol. The production of butyrate or butanol proceeds via acetoacetyl-CoA that is formed from two acetyl-CoA molecules (Figure 1). Products that can be formed from H_2/CO_2 are thus limited to those that allow conservation of sufficient metabolic energy, unless an additional energy substrate is provided.

Hydrogenogenic carboxydotrophs conserve metabolic energy through the formation of H_2. In these microorganisms CO is oxidised by a monofunctional CO dehydrogenase. Electrons released by the oxidation are transferred to an energy converting hydrogenase (ECH) that reduces protons to molecular hydrogen (Figure 2) [28,29]. In addition, ECH couples the formation of H_2 to the membrane translocation of protons or sodium ions (Figure 2), generating a chemiosmotic ion gradient that can drive ATP synthesis through an ATP-synthase [28,30]. Energy conservation in carboxydotrophic hydrogenogenic microorganisms is thus independent of the acetyl-CoA pathway. However, it is expected that most thermophilic carboxydotrophic hydrogenogens contain the acetyl-CoA pathway for carbon fixation, while the currently known mesophilic strains employ a different route (Table 1).

Metabolic engineering

In the carboxydotrophic hydrogenogenic metabolism of, for example, *C. hydrogenoformans*, CO serves as the carbon source, electron donor, and energy source. Oxidation of CO to CO_2 is indirectly coupled to ATP formation and provides reducing equivalents for the reduction of ferredoxin or $NAD(P)^+$ [28,31]. The bacterium can thus easily balance the generation of ATP and the formation of reducing equivalents to fit needs for optimal growth. In sugar-fermenting bacteria, reducing equivalents and ATP are formed in more or less fixed ratios. Metabolic

Figure 2



Schematic representation of CO oxidation, electron transfer, H_2 production and proton translocation by the membrane-bound CO oxidizing: H_2-evolving enzyme complex of carboxydotrophic hydrogenogens, as proposed by Hedderich [28]. CODH, CO dehydrogenase; Fd, ferredoxin; ECH, energy-conserving hydrogenase.

engineering of these organisms with the aim of producing of a specific compound can thus be accompanied by the formation of undesired byproducts, which are formed to satisfy the redox balance [32,33]. Additional separation techniques are then required to obtain a purified product. These disadvantages do not seem to apply for syngas-fermenting microorganisms. Although H_2 is produced as a byproduct, it is easily separated from the aqueous phase through its low solubility. Furthermore, H_2 is not a waste product, but has many uses. Another advantage of using syngas over dissolved sugars as feedstock is that that the use of a gaseous substrate allows the uncoupling of the hydraulic retention time from the substrate supply. This offers possibilities to control substrate inhibition and product formation.

Conclusions

Syngas fermentation is an attractive technology for the production of biofuels and chemicals. A process for ethanol production from syngas is already available, and pure H_2 production is possible as well. At present, suitable thermophiles for the production of organic compounds from syngas are not available, although their use could offer potential advantages over the use of mesophiles. Thermophiles that employ CO as a substrate for the production of chemicals could be selected based on the identification of CO dehydrogenase genes in their genome. Better still would be the isolation of new thermophiles that use CO or syngas as a substrate at conditions that resemble expected bioreactor conditions.

Currently known syngas-fermenting bacteria produce H_2, formate, acetate, ethanol, butyrate and/or butanol. In addition to short-chain organic molecules, the synthesis of long-chain fatty acids and alcohols from syngas by natural isolates might also be possible. The product range of syngas fermentations might be further broadened by metabolic engineering of hydrogenogenic carboxydotrophs. However, metabolic engineering remains highly speculative at present, as no genetic tools are currently available that allow the manipulation of thermophilic CO-converting anaerobes.

Acknowledgements

Our research on CO-converting anaerobes was made possible by grants of Stichting Technologische Wetenschappen (STW), the applied science division of the Nederlandse organisatie voor Wetenschappelijk Onderzoek (NWO, The Netherlands), and financial support from Shell Global Solutions (Amsterdam, The Netherlands) and Paques B.V. (Balk, The Netherlands).

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. • Ragauskas AJ, Williams CK, Davison BH, Britovsek G, Cairney J, Eckert CA, Frederick WJ Jr, Hallett JP, Leak DJ, Liotta CL *et al.*: **The path forward for biofuels and biomaterials**. *Science* 2006, **311**:484-489.
Review that indicates the specific niches of the different biomass processing technologies in future biomaterials and biofuels production. Anaerobic fermentation of syngas is considered a promising and competing technology.

2. Huber GW, Iborra S, Corma A: **Synthesis of transportation fuels from biomass: chemistry, catalysts, and engineering**. *Chem Rev* 2006, **106**:4044-4098.

3. Gosselink JW: **Pathways to a more sustainable production of energy: sustainable hydrogen — a research objective for Shell**. *Int J Hydrogen Energy* 2002, **27**:1125-1129.

4. Demirbas A: **Progress and recent trends in biofuels**. *Progress in Energy and Combustion Science* 2007, **33**:1-18.

5. Newsome DS: **Water-gas shift reaction**. *Catalysis Rev: Sci Eng* 1980, **21**:275-281.

6. Williamson C: **The energy sector: a hidden goliath**. In *Primary Wood Processing*. Edited by Walker JCF. Netherlands: Springer; 2006:535-556.

7. Van Kasteren JMN: **Co-gasification of wood and polyethylene with the aim of CO and H_2 production**. *J Mat Cycles Waste Management* 2006, **8**:95-98.

8. Bredwell MD, Srivastava P, Worden RM: **Reactor design issues for synthesis-gas fermentations**. *Biotechnol Prog* 1999, **15**:834-844.

9. Klasson KT, Ackerson CMD, Clausen EC, Gaddy JL: **Biological conversion of synthesis gas into fuels**. *Int J Hydrogen Energy* 1992, **17**:281.

10. Steele BCH, Heinzel A: **Materials for fuel-cell technologies**. *Nature* 2001, **414**:345-352.

11. Ebrahimi S, Picioreanu C, Xavier JB, Kleerebezem R, Kreutzer M, Kapteijn F, Moulijn JA, van Loosdrecht MCM: **Biofilm growth pattern in honeycomb monolith packings: Effect of shear rate and substrate transport limitations**. *Catal Today* 2005, **105**:448-454.

12. Nerenberg R, Rittmann BE: **Hydrogen-based, hollow-fiber membrane biofilm reactor for reduction of perchlorate and other oxidized contamitants**. *Water Sci Technol* 2004, **49**:223.

13. Rittmann BE, Nerenberg R, Lee KC, Najm I, Gillogly TE, Lehman GE, Adham SS: **Hydrogen-based hollow-fiber membrane biofilm reactor (MBfR) for removing oxidized contaminants**. *Water Sci Technol: Water Supply* 2004, **4**:127.

14. Madigan MT, Martinko JM, Parker J: In *Biology of Microorganisms,* Edn 8. Edited by Brock TD. New Jersey: Prentice-Hall, Inc.; 1997.

15. • Slepova TV, Sokolova TG, Lysenko AM, Tourova TP, Kolganova TV, Kamzolkina OV, Karpov GA, Bonch-Osmolovskaya EA: **Carboxydocella sporoproducens sp. nov., a novel anaerobic CO-utilizing/H_2-producing thermophilic bacterium from a Kamchatka hot spring**. *Int J Syst Evol Microbiol* 2006, **56**:797.
Describes the most recently isolated thermophilic chemolithoautotrophic carboxydotroph that produces H_2. Demonstrates that thermophilic carboxydotrophs can be isolated from natural habitats.

16. • Parshina SN, Sipma J, Nakashimada Y, Henstra AM, Smidt H, Lysenko AM, Lens PNL, Lettinga G, Stams AJM: ***Desulfotomaculum carboxydivorans* sp. nov., a novel sulfate-reducing bacterium capable of growth at 100% CO**. *Int J Syst Evol Microbiol* 2005, **55**:2159-2165.
Describes the isolation of a carboxydotrophic thermophile capable of sulfate reduction. Demonstrates that thermophilic carboxydotrophs can also be isolated from more unexpected, mesophilic habitats such as wastewater treatment plants.

17. Daniel SL, Hsu T, Dean SI, Drake HL: **Characterization of the hydrogen- and carbon monoxide-dependent chemolithotrophic potentials of the acetogens *Clostridium thermoaceticum* and *Acetogenium kivui***. *J Bacteriol* 1990, **172**:4464-4471.

18. Kerby R, Zeikus JG: **Growth of *Clostridium thermoacetium* on H_2/CO_2 or CO as energy source**. *Curr Microbiol* 1983, **8**:27.

19. Savage MD, Wu ZG, Daniel SL, Lundie LL, Drake HL: **Carbon monoxide-dependent chemolithotrophic growth of *Clostridium thermoautotrophicum***. *Appl Environ Microbiol* 1987, **53**:1902-1906.

20. Bao Q, Tian Y, Li W, Xu Z, Xuan Z, Hu S, Dong W, Yang J, Chen Y, Xue Y *et al.*: **A complete sequence of the *T. tengcongensis* genome**. *Genome Res* 2002, **12**:689-700.

21. Klenk HP, Clayton RA, Tomb JF, White O, Nelson KE, Ketchum KA, Dodson RJ, Gwinn M, Hickey EK, Peterson JD *et al.*: **The complete genome sequence of the hyperthermophilic, sulphate-reducing archaeon *Archaeoglobus fulgidus***. *Nature* 1997, **390**:364-370.

22. • Henstra AM, Dijkema C, Stams AJM: ***Archaeoglobus fulgidus* couples CO oxidation to sulfate reduction and acetogenesis with transient formate accumulation.** *Environ Microbiol* 2007, in press.
First report of CO-dependent organic acid production by a hyperthermophilic microorganism. *Archaeoglobus fulgidus* was selected for growth studies with CO on the basis of whole genome sequence information.

23. Lindahl PA: **The Ni-containing carbon monoxide dehydrogenase family: light at the end of the tunnel?** *Biochemistry* 2002, **41**:2097-2105.

24. Ragsdale SW: **Life with carbon monoxide**. *Crit Rev Biochem Mol Biol* 2004, **39**:165-195.

25. Svetlitchnyi V, Dobbek H, Meyer-Klaucke W, Meins T, Thiele B, Romer P, Huber R, Meyer O: **A functional Ni-Ni-[4Fe-4S] cluster in the monomeric acetyl-CoA synthase from *Carboxydothermus hydrogenoformans***. *Proc Natl Acad Sci USA* 2004, **101**:446-451.

26. Wu M, Ren Q, Durkin AS, Daugherty SC, Brinkac LM, Dodson RJ, Madupu R, Sullivan SA, Kolonay JF, Nelson WC *et al.*: **Life in hot carbon monoxide: the complete genome sequence of *Carboxydothermus hydrogenoformans* Z-2901**. *PLoS Genetics* 2005, **1**:e65.

27. Drake HL: *Acetogenesis*. New York: Chapman and Hall; 1994.

28. Hedderich R: **Energy-converting [NiFe] hydrogenases from archaea and extremophiles: ancestors of complex I**. *J Bioenerg Biomembr* 2004, **36**:65-75.

29. Singer SW, Hirst MB, Ludden PW: **CO-dependent H_2 evolution by *Rhodospirillum rubrum*: role of CODH:CooF complex**. *Biochim Biophys Acta - Bioenerg* 2006, **1757**:1582-1591.

30. Maness PC, Huang J, Smolinski S, Tek V, Vanzin G: **Energy generation from the CO oxidation-hydrogen production pathway in *Rubrivivax gelatinosus***. *Appl Environ Microbiol* 2005, **71**:2870-2874.

31. Svetlitchnyi V, Peschel C, Acker G, Meyer O: **Two membrane-associated NiFeS-carbon monoxide dehydrogenases from the anaerobic carbon-monoxide-utilizing eubacterium *Carboxydothermus hydrogenoformans***. *J Bacteriol* 2001, **183**:5134-5144.

32. Holms H: **Flux analysis and control of the central metabolic pathways in *Escherichia coli***. *FEMS Microbiol Rev* 1996, **19**:85-116.

33. Yun NR, San KY, Bennett GN: **Enhancement of lactate and succinate formation in *adhE* or *pta-ackA* mutants of NADH dehydrogenase-deficient *Escherichia coli***. *J Appl Microbiol* 2005, **99**:1404-1412.

34. Abrini J, Naveau H, Nyns EJ: ***Clostridium autoethanogenum,* sp. nov., an anaerobic bacterium that produces ethanol from carbon monoxide**. *Arch Microbiol* 1994, **161**:345-351.

35. Tanner RS, Miller LM, Yang D: ***Clostridium ljungdahlii* sp. nov., an acetogenic species in clostridial ribosomal-RNA homology group-I**. *Int J Syst Bacteriol* 1993, **43**:232-236.

36. Liou JSC, Balkwill DL, Drake GR, Tanner RS: ***Clostridium carboxidivorans* sp. nov., a solvent-producing clostridium isolated from an agricultural settling lagoon, and reclassification of the acetogen *Clostridium scatologenes* strain SL1 as *Clostridium drakei* sp. nov**. *Int J Syst Evol Microbiol* 2005, **55**:2085-2091.

37. Krumholz LR, Bryant MP: ***Clostridium pfennigii* sp. nov. uses methoxyl groups of monobenzenoids and produces butyrate**. *Int J Syst Bacteriol* 1985, **35**:454-456.

38. Lorowitz WH, Bryant MP: ***Peptostreptococcus productus* strain that grows rapidly with CO as the energy source**. *Appl Environ Microbiol* 1984, **47**:961-964.

39. Sharak Genthner BR, Bryant MP: **Additional characteristics of one-carbon-compound utilization by *Eubacterium limosum* and *Acetobacterium woodii***. *Appl Environ Microbiol* 1987, **53**:471-476.

40. Sharak Genthner BR, Bryant MP: **Growth of *Eubacterium limosum* with carbon monoxide as the energy source**. *Appl Environ Microbiol* 1982, **43**:70-74.

41. Grethlein AJ, Worden RM, Jain MK, Datta R: **Evidence for production of *n*-butanol from carbon monoxide by *Butyribacterium methylotrophicum***. *J Ferment Bioeng* 1991, **72**:58-60.

42. Lynd L, Kerby R, Zeikus JG: **Carbon monoxide metabolism of the methylotrophic acidogen *Butyribacterium methylotrophicum***. *J Bacteriol* 1982, **149**:255-263.

43. Shen GJ, Shieh JS, Grethlein AJ, Jain MK, Zeikus JG: **Biochemical basis for carbon monoxide tolerance and butanol production by *Butyribacterium methylotrophicum***. *Appl Microbiol Biotechnol* 1999, **51**:827-832.

44. Dashekvicz MP, Uffen RL: **Identification of a carbon monoxide metabolizing bacterium as a strain of *Rhodopseudomonas gelatinosa* (Molisch) Vanniel**. *Int J Syst Bacteriol* 1979, **29**:145-148.

45. Uffen RL: **Anaerobic growth of a *Rhodopseudomonas* species in the dark with carbon monoxide as sole carbon and energy substrate**. *Proc Natl Acad Sci USA* 1976, **73**:3298-3302.

46. Jung GY, Jung HO, Kim JR, Ahn Y, Park S: **Isolation and characterization of *Rhodopseudomonas palustris* P4 which utilizes CO with the production of H_2**. *Biotechnol Lett* 1999, **21**:525-529.

47. Kerby RL, Ludden PW, Roberts GP: **Carbon monoxide-dependent growth of *Rhodospirillum rubrum***. *J Bacteriol* 1995, **177**:2241-2244.

48. Jung GY, Kim JR, Jung HO, Park JY, Park S: **A new chemoheterotrophic bacterium catalyzing water-gas shift reaction**. *Biotechnol Lett* 1999, **21**:869-873.

49. Jung GY, Kim JR, Park JY, Park S: **Hydrogen production by a new chemoheterotrophic bacterium *Citrobacter sp*. Y19**. *Int J Hydrogen Energy* 2002, **27**:601-610.

50. O'Brien JM, Wolkin RH, Moench TT, Morgan JB, Zeikus JG, Lupton FS, Conrad R: **Association of hydrogen metabolism with unitrophic or mixotrophic growth of *Methanosarcina barkeri* on carbon monoxide**. *J Bacteriol* 1984, **158**:373-375.

51. Rother M, Metcalf WW: **Anaerobic growth of *Methanosarcina acetivorans* C2A on carbon monoxide: an unusual way of life for a methanogenic archaeon**. *Proc Natl Acad Sci USA* 2004, **101**:16929-16934.

52. Sokolova TG, Gonzalez JM, Kostrikina NA, Chernyh NA, Tourova TP, Kato C, Bonch Osmolovskaya EA, Robb FT: ***Carboxydobrachium pacificum* gen. nov., sp nov., a new anaerobic, thermophilic, CO-utilizing marine bacterium from Okinawa Trough**. *Int J Syst Evol Microbiol* 2001, **51**:141-149.

53. Sokolova TG, Kostrikina NA, Chernyh NA, Tourova TP, Kolganova TV, Bonch-Osmolovskaya EA: ***Carboxydocella thermautotrophica* gen. nov., sp. nov., a novel anaerobic, CO-utilizing thermophile from a Kamchatkan hot spring**. *Int J Syst Evol Microbiol* 2002, **52**:1961-1967.

54. Sokolova TG, Kostrikina NA, Chernyh NA, Kolganova TV, Tourova TP, Bonch-Osmolovskaya EA: ***Thermincola carboxydiphila* gen. nov., sp. nov., a novel anaerobic, carboxydotrophic, hydrogenogenic bacterium from a hot**

spring of the Kae Baikal area. *Int J Syst Evol Microbiol* 2005, **55**:2069-2073.

55. Zavarzina DG, Sokolova TG, Tourova TP, Chernyh NA, Kostrikina NA, Bonch-Osmolovskaya EA: ***Thermincola ferriacetica* sp. nov., a new anaerobic, thermophilic, facultatively chemolithoautotrophic bacterium capable of dissimilatory Fe(III) reduction**. *Extremophiles* 2007, **11**:1.

56. Svetlichnyi VA, Sokolova TG, Kostrikina NA, Lysenko AM: ***Carboxydothermus restrictus* sp. nov. - a new thermophilic anaerobic carboxydotrophic bacterium**. *Mikrobiologiya* 1994, **63**:523-528.

57. Sokolova T, Hanel J, Onyenwoke RU, Reysenbach AL, Banta A, Geyer R, Gonza JM, Whitman WB, Wiegel J: **Novel chemolithotrophic, thermophilic, anaerobic bacteria *Thermolithobacter ferrireducens* gen. nov., sp. nov. and *Thermolithobacter carboxydivorans* sp. nov**. *Extremophiles* 2007, **11**:145.

58. Sokolova TG, Gonzalez JM, Kostrikina NA, Chernyh NA, Slepova TV, Bonch-Osmolovskaya EA, Robb FT: ***Thermosinus carboxydivorans* gen. nov., sp. nov., a new anaerobic, thermophilic, carbon monoxide-oxidizing, hydrogenogenic bacterium from a hot pool of Yellowstone National Park**. *Int J Syst Evol Microbiol* 2004, **54**:2353-2359.

59. Parshina SN, Kijlstra S, Henstra AM, Sipma J, Plugge CM, Stams AJM: **Carbon monoxide conversion by thermophilic sulfate-reducing bacteria in pure culture and in co-culture with *Carboxydothermus hydrogenoformans***. *Appl Microbiol Biotechnol* 2005, **68**:390-396.

60. Daniels L, Fuchs G, Thauer RK, Zeikus JG: **Carbon monoxide oxidation by methanogenic bacteria**. *J Bacteriol* 1977, **132**:118-126.

61. Sokolova TG, Jeanthon C, Kostrikina NA, Chernyh NA, Lebedinsky AV, Stackebrandt E, Bonch-Osmolovskaya EA: **The first evidence of anaerobic CO oxidation coupled with H_2 production by a hyperthermophilic archaeon isolated from a deep-sea hydrothermal vent**. *Extremophiles* 2004, **8**:317-323.