WHO MAKES THE METHANE

The three routes to methane in a digester, and the organisms that carry them

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ABSTRACT

A companion to *What one digestate sample can tell you*. A digester report names methanogens by genus and says which route to methane each one carries. This note explains the three routes, which organisms carry each, and what the literature says their presence tends to mean, so that the names in a report can be read rather than looked up.

THREE SUBSTRATES, ONE LAST STEP

Every litre of biomethane is made by archaea, and every methanogen finishes the job the same way: a methyl group bound to coenzyme M is reduced to methane by methyl-coenzyme M reductase, the enzyme encoded by *mcrA* (Thauer et al. 2008; Evans et al. 2019). What differs between them is what they feed into that last step. There are three substrate groups, and they give the three routes that a digester report names: carbon dioxide reduced with hydrogen (or formate), acetate, and methylated compounds such as methanol and methylamines (Liu and Whitman 2008).

The routes are not interchangeable, and they are not carried by the same organisms. For sewage-sludge digesters at low ammonia, the textbook estimate is that about two thirds of the methane comes from acetate and most of the rest from hydrogen and

carbon dioxide (Demirel and Scherer 2008). In ammonia-rich agricultural reactors the balance is often reversed, for reasons the section on acetate below explains. When a plant is under stress the organisms carrying each route change, and that is what DNA can see.

mcrA is the marker a report uses to decide whether a genome is a methanogen at all. Anaerobic methane oxidisers carry it too and run the pathway in reverse, which is why the genus matters as much as the gene.

THE HYDROGEN ROUTE

Hydrogenotrophic methanogens reduce CO_2 to methane with hydrogen, and many can use formate instead. They are the most widespread methanogens and span several orders: *Methanobacteriales* (*Methanobacterium*, *Methanothermobacter*, *Methanobrevibacter*), *Methanomicrobiales* (*Methanoculleus*, *Methanocorpusculum*), *Methanococcales* and *Methanocellales* among them (Liu and Whitman 2008).

Their role in a digester is larger than their share of the methane. The bacteria that break down propionate and butyrate release hydrogen, and they can only keep doing so while hydrogen is removed as fast as it forms. Hydrogenotrophs are the organisms that remove it (Demirel and Scherer 2008). When they fall behind, volatile fatty acids accumulate: the familiar first sign of an upset has a hydrogen consumer behind it.

Methanoculleus deserves a name of its own. It is repeatedly found as the dominant hydrogen consumer of ammonia-rich digesters, where its tolerance of ammonia is part of why it persists as other methanogens decline (Westerholm, Moestedt, and Schnürer 2016; Wang, Fotidis, and Angelidaki 2015), and *Methanoculleus bourgensis* has been isolated from a biogas reactor (Maus et al. 2016). In the example report it carries the hydrogen route in both plants. In the BP3 reactor behind the depth tests

in *What one digestate sample can tell you*, it was the only methanogen assembled at every depth from 2.5 to 12 Gb.

THE ACETATE ROUTE

Only two groups of methanogens split acetate: *Methanosarcina* and the *Methanotrichaceae*: *Methanothrix* (formerly *Methanosaeta*) and *Methanocrinis*, which now holds the former *Methanothrix harundinacea*, a digester organism, alongside a newly described alkaliphilic species (Smith and Ingram-Smith 2007; Khomyakova et al. 2023). Because the route is carried by so few genera, which of them carries it in a given reactor says a good deal about that reactor.

Methanothrix is the specialist. It uses nothing but acetate, grows slowly, and works at very low acetate concentrations that *Methanosarcina* cannot use (Jetten, Stams, and Zehnder 1992; Smith and Ingram-Smith 2007). It wins in a stable, well-fed reactor where acetate never accumulates. *Methanosarcina* is the generalist. It needs more acetate, grows faster, can use methyl compounds and, in most species, hydrogen, and tolerates high acetate, ammonia and salt far better, which has earned it the description of a methanogen for heavy-duty biomethanation (De Vrieze et al. 2012; Oren 2014). A survey of full-scale digesters found *Methanothrix* absent from reactors with high ammonia and volatile fatty acids, and *Methanosarcina* dominant in them (Karakashev, Batstone, and Angelidaki 2005).

That is the distinction the example report turns on. Plant A carries its acetate route on *Methanothrix soehngenii* at 3.7 % of mapped reads: abundant, efficient, and sensitive to ammonia. Plant B carries it

Several relatives of *Methanosarcina* (*Methanolobus*, *Methanococcoides*, *Methanomethylovorans*) carry much of the same machinery but do not grow on acetate. A report credits the acetate route by genus, not by gene content alone, for that reason.

on *Methanosarcina flavescens* at 0.49 %: scarce, but of a genus that tolerates what would stop plant A's.

WHEN ACETATE TAKES THE LONG WAY ROUND

Under high ammonia the acetate splitters are inhibited, and acetate does not simply accumulate. Syntrophic bacteria oxidise it to hydrogen and CO_2, and hydrogenotrophic methanogens turn those into methane (Schnürer and Nordberg 2008; Westerholm, Moestedt, and Schnürer 2016). The methane still comes from acetate, but through the hydrogen route, and the partnership depends on hydrogen being kept low, so it is slower and less forgiving than splitting acetate directly (Westerholm, Moestedt, and Schnürer 2016).

A community whose acetate route is thin and whose methanogens are mostly *Methanoculleus* is therefore consistent with a reactor running this way. DNA shows that the organisms and genes for it are present. It does not show that the pathway is carrying the flux, and a report says the first and not the second.

THE METHYL ROUTE

Methylotrophic methanogens make methane from methanol, methylamines and methylated sulfides (Söllinger and Urich 2019). In a digester these come from the feed: methanol from the demethylation of pectin, methylamines from the breakdown of choline, betaine and similar compounds (Söllinger and Urich 2019). The route is usually a minor share of the methane, but the organisms carrying it are more varied than for either of the other two.

Two kinds are worth telling apart. Some *Methanosarcinales* split methyl compounds on their own, among them *Methanosarcina* itself and the obligately methylotrophic *Methanolobus*, *Methanococcoides* and *Methanomethylovorans* (Oren 2014). Others reduce methyl groups with hydrogen and cannot make methane any other way: the *Methanomassiliicoccales* (Lang et al. 2015), *Methanosphaera* (Fricke et al. 2006), the *Methanofastidiosa* (Nobu et al. 2016) and the *Verstraetearchaeota* (Vanwonterghem et al. 2016). These consume hydrogen but cannot reduce CO_2, so a report lists them under the methyl route; they still compete with the hydrogenotrophs for hydrogen. *Methanogranum*, which carries the methyl route in plant A of the example report, is one of the *Methanomassiliicoccales*.

Methylamine use depends on pyrrolysine, an amino acid encoded by a codon that normally means “stop”. Gene-calling software that stops there splits the gene in two, which is one reason the analysis calls its own genes.

READING THE NAMES IN A REPORT

GENUS	ROUTE	WHAT IT TENDS TO MEAN
Methanothrix	acetate	stable reactor, low acetate; sensitive to ammonia
Methanocrinis	acetate	the former *Methanothrix harundinacea*; a digester acetate specialist
Methanosarcina	acetate, methyl, most species hydrogen	tolerant of acetate, ammonia and salt; grows fast
Methanoculleus	hydrogen	common in ammonia-rich reactors; partner of acetate oxidisers
Methanobacterium, *Methanothermobacter*	hydrogen	hydrogen consumers; the latter thermophilic
Methanocorpusculum, *Methanospirillum*	hydrogen	hydrogen consumers
Methanomassiliicoccales (e.g. *Methanogranum*)	methyl, with hydrogen	methyl compounds in the feed

Table 1: Methanogens a digester report is likely to name, the route each carries, and the usual reading of its presence. Tendencies, not rules.

A single name rarely decides anything. The useful reading is the combination: which genus carries each route, how much of it there is, and whether a route rests on one genome or several. A reactor whose acetate route rests on one ammonia-sensitive genome is in a different position from one where it rests on a tolerant genus, even if both are producing the same gas today.

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