Formation and Fate of Fermentation Products in Hot Spring Cyanobacterial Mats

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The fate of representative fermentation products (acetate, propionate, butyrate, lactate, and ethanol) in hot spring cyanobacterial mats was investigated. The major fate during incubations in the light was photoassimilation by filamentous bacteria resembling Chloroflexus aurantiacus. Some metabolism of all compounds occurred under dark aerobic conditions. Under dark anaerobic conditions, only lactate was oxidized extensively to carbon dioxide. Extended preincubation under dark anaerobic conditions did not enhance anaerobic catabolism of acetate, propionate, or ethanol. Acetogenesis of butyrate was suggested by the hydrogen sensitivity of butyrate conversion to acetate and by the enrichment of butyrate-degrading acetogenic bacteria. Accumulation of fermentation products which were not catabolized under dark anaerobic conditions revealed their importance. Acetate and propionate were the major fermentation products which accumulated in samples collected at temperatures ranging from 50 to 70°C. Other organic acids and alcohols accumulated to a much lesser extent. Fermentation occurred mainly in the top 4 mm of the mat. Exposure to light decreased the accumulation of acetate and presumably of other fermentation products. The importance of interspecies hydrogen transfer was investigated by comparing fermentation product accumulation at a 65°C site, with naturally high hydrogen levels, and a 55°C site, where active methanogenesis prevented significant hydrogen accumulation. There was a greater relative accumulation of reduced products, notably ethanol, in the 65°C mat.

Hot spring microbial mats provide a natural system in which organic matter is formed and decomposed by thermophilic microorganisms, some of which may be useful in industrial processes (43, 47). Extreme high temperature restricts diversity (6), simplifying the community and thus studies of its structure and function. Temperature is, however, not so extreme as to interrupt normal elemental cycles (37, 39), so that these mats may be models for other more complex aquatic communities. Hot spring mats are also considered modern analogs of Precambrian microbial communities which are preserved as stromatolites (1, 8, 35). Understanding such modern communities should improve our knowledge of Precambrian life.

The cyanobacterial mats of neutral-alkaline springs, such as Octopus Spring in Yellowstone National Park (6), are becoming increasingly well understood. A single cyanobacterium, Synechococcus lividus, which occupies the top 1 mm in the mat, is responsible for mat formation and dramatically influences the oxygen, sulfide, and pH chemistry of the mat by its photosynthesis (6, 27). The photosynthetic bacterium Chloroflexus aurantiacus, which in culture prefers to grow as a phototrophotroph, resides within, and in the 2 to 3 mm beneath, the cyanobacterial layer (3, 6). Photosynthesis is balanced by decomposition, so that the mat is in a steady state (9).

Studies on decomposition have mainly focused on terminal anaerobic processes (37, 39). The occurrence of methanogenesis (29, 30, 36) and sulfate reduction (37, 38) implies that, as in other anaerobic environments, there is a link between microorganisms which ferment polymeric organic substrates and microbial groups which terminate anaerobic food chains and consume fermentation products (37, 40).

Although several fermentative bacteria have been isolated from hot springs (25, 28) and hot spring cyanobacterial mats (4, 31, 41, 42, 44-46, 48, 49), little is known about fermentation processes in these mats. In many other anaerobic systems, acetogenic bacteria play an important role in conversion of reduced fermentation products to the substrates of methanogenic bacteria (21). The importance of acetogens in anaerobic decomposition in hot spring microbial mats is of particular interest, since our previous studies suggested that C. aurantiacus may interrupt the normal catabolism of organic fermentation products by chemotrophic anaerobes. For example, acetate, usually a major substrate for methanogenesis, was not catabolized during dark anaerobic incubations, but was readily photoassimilated, leaving H2 as the exclusive energy source for methanogenesis (29). If C. aurantiacus interrupts the cycling of reduced organic fermentation products, this genus may also replace acetogenic bacteria in controlling the fate of such fermentation products.

In this study we investigated the formation and fate of fermentation products in hot spring cyanobacterial mats. We were especially interested in the roles of C. aurantiacus and acetogenic bacteria in controlling the fate of various potential fermentation products and in the dependence of fermentation and acetogenesis on product consumption by methanogenic bacteria.

MATERIALS AND METHODS

Study areas. The major study area was Octopus Spring, a silicic, slightly alkaline (pH 8.3) spring located in the Lower Geyser Basin of Yellowstone National Park (9). Cyanobacterial mat samples were collected from sites where
temperatures averaged 50, 55, 60, 65, or 70°C. Some experiments were performed on mat samples collected at a 55°C region in Mushroom Spring, also located in the Lower Geyser Basin (6).

Sampling and treatment of samples. Samples removed from the mat with a no. 4 or no. 6 cork borer (top 1 cm by 50.3 or 78.5 mm², respectively) were placed in glass vials, which were then capped with butyl rubber stoppers, under a headspace of either air, nitrogen (fermentation product accumulation studies), or helium (radio-labeling studies), as described previously (29). Spring water (1 to 2 mm) from above the mat (or, in anaerobic incubations, spring water made anoxic by bubbling with helium or nitrogen) was added to each sample. Dark conditions were simulated by wrapping vials with several layers of black tape.

In depth profile studies, replicate 1-cm-long cores were sectioned with a razor blade into approximate vertical intervals (top 1 mm and 0 to 2, 0 to 4, 0 to 6, 0 to 8, and 0 to 10 mm) and were treated as specified above.

In some experiments methanogenesis was inhibited by addition of 2-bromoethanesulfonic acid (BES) (10) from an anoxic stock solution (to 50 mM) in order to force hydrogen accumulation. It was previously determined that this concentration caused complete inhibition of methanogenesis (T. A. Tayne, M. S. thesis, Montana State University, Bozeman, 1983).

Radio-labeling experiments. In short-term experiments, samples were preincubated in the effluent channel at 50°C for 30 min before addition of radiolabeled compounds. To intentionally enrich for aceticogenic activity, some samples were preincubated under dark anaerobic conditions for 24 h. For extended incubations, samples were held within 5°C of the in situ temperature in a Thermost during transport to the laboratory and then transferred to a dark 50°C incubator. After the desired preincubation, radiolabeled compounds were added to approximately 1 µCi per core from 10-fold-concentrated sterile stock solutions. Following incubation, 0.1 ml of Formalin was added, and samples were dispensed by shaking to stop biological activity.

The radioactive compounds used were [2-14C]acetic acid (51 mCi/mmole; New England Nuclear Corp.), [1-14C]butyric acid (52 to 56 mCi/mmole; Amersham Corp.), [1-14C]propiolic acid (56.7 to 58.4 mCi/mmole; New England Nuclear), [1-14C]ethanol (21 mCi/mmole; New England Nuclear), and DL-[1-14C]acetic acid (51 mCi/mmole; Amersham).

Enrichment of aceticogenic bacteria. The medium for enrichment of aceticogenic bacteria in coculture with methanogenic bacteria was that used by McInerney et al. (22), except that medium D (7) was substituted for Pfennig medium. Anaerobic conditions were established as described by Hungate (14). Sodium propionate (to 18 mM), n-butyrate (to 18 mM), or ethanol (to 20 mM) was added from concentrated anoxic stock solutions. Controls with no added carbon and energy source were inoculated simultaneously. Tubes of medium (10 ml) were inoculated in the field by adding anoxically a core (1 cm by 50.3 mm²) from the 50°C mat at Octopus Spring. Before the tube was sealed under N₂, Na₂S: 9H₂O was added as a reducing agent to a final concentration of 0.05%. The final pH was 8.2. Enrichments were transported to the lab as described above and then transferred to a 30°C dark incubator. They were checked every few weeks for turbidity and by gas headspace analysis for methane production. Once each month 1 ml of each positive enrichment culture (methane above control) was transferred to a fresh tube containing the same substrate.

Fermentation product accumulation. Samples were trans-
are reported as percent in fraction to minimize error associated with small-volume additions of radiolabeled compounds. Means of triplicate samples were compared by a two-sample t test (19).

Autoradiography of 14C-labeled homogenates was done as described by Sandbeck and Ward (29). A Leitz Ortholux II microscope with interference contrast optics was used to evaluate autoradiograms.

14C-volatile fatty acids were analyzed by GC-GPC after concentrating filtrate samples. Filtrate (1 ml) was mixed with 20 μl of 50 mM hexanoic acid (as an internal standard) and 100 μl of 2 N NaOH in a 2-ml Eppendorf centrifuge tube. Samples were incubated at 60°C until dry (ca. 24 h) and then rehydrated in 200 μl of 0.25 M m-phosphoric acid. This technique concentrated the original sample five times with no detectable loss of volatile fatty acids, as determined by concentration of standard solutions. Samples were then injected into the GC system used for volatile fatty acid analysis (as above) coupled via a stainless steel stream splitter by a Teflon line (3 mm outer diameter, heated to 150°C) to the combustion furnaces of the GPC. The flow to the GPC was increased to 21 ml/min. with helium makeup gas added after the splitter. The operating conditions of the GPC have been described (38). Standard curves were prepared to relate the area unit response of the GPC to the disintegrations of 14C-volatile fatty acids injected, as determined by liquid scintillation counting of standards concentrated in the same way.

RESULTS

Fate of fermentation products. The results of a short-term (2-h) incubation of various 14C-labeled fermentation products with samples from the Octopus Spring mat are presented in Table 1. The major fate of all compounds in the light was incorporation into cells, as opposed to oxidation to 14CO2. Significantly less 14C was recovered in cells when samples were incubated in the dark. Under dark aerobic conditions acetate, propionate, ethanol, and lactate were partially metabolized to CO2. Anaerobically in the dark, and especially after dark anaerobic preincubation, fermentation products were not significantly metabolized to 14CO2 or 14CH4 (none detected). Under these conditions radioactivity was recovered mainly in the filtrate. An exception was [1-14C]lactate, which was metabolized to 14CO2 under all conditions.

Since the principal fate of all compounds in both mat types was photoassimilation, autoradiograms of 14C-labeled Octopus Spring mat samples were prepared to examine the morphology of cells responsible for photoassimilation. All fermentation products were assimilated by filamentous cells (Fig. 1).

Potential for acetogenesis. The potential for anaerobic catabolism of fermentation products was investigated following long-term dark anaerobic incubation of Octopus Spring mat samples. Methanogenesis during the incubation period indicated that anaerobic decomposition was occurring (Fig. 2). Inhibition of methanogenesis with BES led to accumulation of H2. By investigating the fate of 14C-labeled fermentation products in control and inhibited samples, the sensitivity of the metabolism of each compound to the accumulation of H2 could be evaluated (Table 2). [1-14C]lactate served as a control, since its metabolism is theoretically independent of H2 concentration; this was confirmed experimentally (Table 2). [1-14C]butyrate, [1-14C]propionate, and [1-14C]ethanol were converted to 14CO2 in small amounts. The conversion of [1-14C]propionate to 14CO2 was sensitive to BES, suggesting the possibility of acetogenic activity. Oxidation of [1-14C]butyrate and [1-14C]ethanol to 14CO2 was not inhibited by BES; however, these compounds should be catabolized mainly to 14C acetate by acetogenic bacteria. Thus, concentrated filtrate samples from this experiment were analyzed for 14C-volatile fatty acids. Figure 3 shows the GPC recorder tracings for analyses of the filtrates from samples incubated with [1-14C]butyrate in the presence and absence of BES. The control sample contained both [14C]acetate and [14C]butyrate, whereas samples with added BES contained only [14C]butyrate, suggesting a potential for acetogenic conversion of butyrate to acetate. Filtrates from samples incubated with [1-14C]propionate or [1-14C]ethanol did not contain [14C]acetate.

The incubation times of the previous experiment were very long (64 h of preincubation followed by 12 h of incubation with radiolabel), so that results do not reflect the importance of acetogenesis under natural conditions. Thus, filtrates from samples incubated for 2 h (from the experiment in Table 1) were analyzed. [14C]acetate was only detected in
labeling experiments with [1-14C]butyrate. As shown in Table 3, in the light no conversion of butyrate to acetate occurred. In the dark, especially in samples incubated anaerobically, metabolism of butyrate to acetate occurred. There was a lower percent conversion in samples which were preincubated for 24 h under dark anaerobic conditions than for samples preincubated for only 30 min prior to the addition of radiolabel.

Enrichment cultures for propionate-, ethanol-, or butyrate-degrading acetogenic bacteria were inoculated with sam-
Fermentation products to accumulate. Acetate accumulated in a ratio of approximately 3 to 1 relative to propionate. Although n-butyrate, iso-butyrate, n-valerate, and iso-valerate also accumulated, these products reached much lower concentrations. This trend was repeated in a number of similar experiments. There was no evidence that nonvolatile fatty acids or alcohols accumulated during an incubation period of up to 120 h (data not shown). Formalin controls showed no accumulation of volatile or nonvolatile fatty acids or alcohols.

Acetate and propionate were major fermentation products to accumulate in samples from 50, 55, 60, 65, and 70°C sites. Large differences in the rates of accumulation of the major fermentation product acetate at different temperatures were not observed.

The vertical position in which fermentation occurs in the

![Graph](image-url)

**FIG. 2.** Effect of BES on methane and hydrogen production during anaerobic incubation of 50°C Octopus Spring cyanobacterial mat samples. Bars, SE.

**TABLE 2.** Effect of BES on the fate of 14C-labeled fermentation products during extended dark anaerobic incubation of 50°C Octopus Spring cyanobacterial mat samples

<table>
<thead>
<tr>
<th>Substrate and compound</th>
<th>Incubation conditions</th>
<th>% of label* in:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cells</td>
</tr>
<tr>
<td>Potential acetogen substrates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[1-14C]propionate</td>
<td>Control</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>BES</td>
<td>0.18</td>
</tr>
<tr>
<td>[1-14C]butyrate</td>
<td>Control</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>BES</td>
<td>0.20</td>
</tr>
<tr>
<td>[1-14C]ethanol</td>
<td>Control</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>BES</td>
<td>0.075</td>
</tr>
<tr>
<td>Nonacetogen substrate ([1-14C]lactate)</td>
<td>Control</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>BES</td>
<td>0.7</td>
</tr>
</tbody>
</table>

* Following 64 h of preincubation with and without BES, as indicated in Fig. 2, samples were incubated for 12 h with radiolabel.

**FIG. 3.** Recorder tracing of GPC response showing the effect of BES on conversion of [1-14C]butyrate to 14Cacetate during anaerobic incubation of 50°C Octopus Spring cyanobacterial mat samples. Samples were preincubated in the presence and absence of BES for 64 h (as shown in Fig. 2) and then for 12 h with [1-14C]butyrate.

**TABLE 3.** Relative accumulation of fermentation products during anaerobic incubation of Octopus Spring cyanobacterial mat samples

<table>
<thead>
<tr>
<th>Substrate and compound</th>
<th>Incubation conditions</th>
<th>% of label* in:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cells</td>
</tr>
<tr>
<td>Potential acetogen substrates</td>
<td></td>
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</tr>
<tr>
<td>[1-14C]propionate</td>
<td>Control</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>BES</td>
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<tr>
<td>[1-14C]butyrate</td>
<td>Control</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>BES</td>
<td>0.20</td>
</tr>
<tr>
<td>[1-14C]ethanol</td>
<td>Control</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>BES</td>
<td>0.075</td>
</tr>
<tr>
<td>Nonacetogen substrate ([1-14C]lactate)</td>
<td>Control</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>BES</td>
<td>0.7</td>
</tr>
</tbody>
</table>

* Following 64 h of preincubation with and without BES, as indicated in Fig. 2, samples were incubated for 12 h with radiolabel.

**Notes:**

- Significant differences (P < 0.05) from control CO2 value.
- ND, None detected.
TABLE 3. Conversion of [1-14C]butyrate to [14C]acetate during short-term (2-h) incubation with samples from the 50°C Octopus Spring cyanobacterial mat

<table>
<thead>
<tr>
<th>Incubation conditions</th>
<th>Mean % of added [14C]butyrate recovered as [14C]acetate ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light Aerobic</td>
<td>ND*</td>
</tr>
<tr>
<td>Anaerobic</td>
<td>ND</td>
</tr>
<tr>
<td>Dark</td>
<td></td>
</tr>
<tr>
<td>Aerobic</td>
<td>7.2 ± 1.7</td>
</tr>
<tr>
<td>Anaerobic</td>
<td>49.1 ± 7.5</td>
</tr>
<tr>
<td>Preincubation†</td>
<td>17.4 ± 1.0</td>
</tr>
</tbody>
</table>

* Filtrates analyzed were from the experiment reported in Table 1. Only filtrates from tubes containing [1-14C]butyrate were found to contain [14C]acetate.
† ND, None detected.
‡ Samples were preincubated for 24 h under dark anaerobic conditions.

55°C Octopus Spring mat was studied in core sections cut to vary in thickness from the top cyanobacterial layer (about the top 1 mm) to 1 cm deep in the mat. Acetate and propionate were again found to be the predominant fermentation products (Fig. 5). Although these fatty acids accumulated in the top cyanobacterial layer, their accumulation was more rapid if the thickness of the mat was increased to 2 or 4 mm. Further increases in thickness did not result in more rapid acetate and propionate accumulation. Similar vertical profiles were observed in incubations as short as 14 h, though the significance of differences was less demonstrable.

Effect of light on fermentation product accumulation. As the potential for fermentation was greatest in the photic zone of the mat, we investigated the influence of light on accumulation of acetate, the easiest product to quantify during short in situ incubations. In cores from a 55°C region of the Octopus Spring mat, acetate accumulated during dark incubation but was consumed in sunlight relative to Formalin controls (Table 4). A similar trend was observed in samples from a 55°C region of the Mushroom Spring mat.

Effect of hydrogen on fermentation product accumulation. The effect of hydrogen on the types of fermentation products produced was investigated by comparing a 65°C site with a 55°C site in the Octopus Spring mat. Hydrogen accumulated as a major gaseous product of anaerobic decomposition at 65°C, whereas methane accumulated at 55°C (Fig. 6). As previously mentioned, acetate and propionate were the major fermentation products at both temperatures (Fig. 7). The accumulation of other volatile fatty acids and ethanol was greater at 65°C. Lactate also accumulated in 65°C but not in 55°C mat samples (data not shown). Although lactate accumulation was statistically significant compared with Formalin controls, the rate of accumulation was very low in comparison with acetate and propionate accumulation.

Because the rates of fermentation at the two sites differed, the relative importance of each fermentation product at either temperature can best be seen after normalizing the amounts of each fermentation product against the amount of acetate, the major fermentation product, which accumulated in the same time interval (Table 5). Thus, ethanol accumulation was 19 times higher at 65°C than at 55°C. Accumulation of other reduced products, such as n-butylate, iso-butylate, and iso-valerate, was also higher at 65°C, but the increased accumulation was more modest (1.34 to 2.76-fold higher than at 55°C). A similar effect was noted when BES was added to 55°C mat samples to inhibit methanogenesis, causing hydrogen to accumulate (as in Fig. 2). Under these conditions, ethanol accumulated to a greater degree than did other reduced fermentation products, but the increase was not as pronounced as in samples of the 65°C mat (3.25-fold higher than at 55°C without inhibition of methanogenesis).

FIG. 4. Accumulation of volatile fatty acids during dark anaerobic incubation of samples of the 50°C Octopus Spring cyanobacterial mat. "Other" refers to the total amount of n- and iso-butyrate and n- and iso-valerate. Bars, SE.

FIG. 5. Depth profile of acetate and propionate accumulation after 54 h of dark anaerobic incubation of samples of the 55°C Octopus Spring cyanobacterial mat. Bars, SE.

TABLE 4. Effect of light on acetate accumulation during dark anaerobic incubation of hot spring cyanobacterial mat samples

<table>
<thead>
<tr>
<th>Sample and temp (°C)</th>
<th>Conditions and incubation time (h)</th>
<th>Collection time</th>
<th>Mean acetate concn* (μmol/vial) ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Octopus Spring, 55</td>
<td>Formalin control</td>
<td>8:35 a.m.</td>
<td>0.95 ± 0.40</td>
</tr>
<tr>
<td></td>
<td>Light (10)</td>
<td>8:35 a.m.</td>
<td>0.04 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>Dark (10)</td>
<td>8:35 a.m.</td>
<td>1.74 ± 0.02*</td>
</tr>
<tr>
<td>Mushroom Spring, 55</td>
<td>Light (6)</td>
<td>12 noon</td>
<td>0.17 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>Dark (6)</td>
<td>12 noon</td>
<td>0.78 ± 0.05*</td>
</tr>
</tbody>
</table>

* *, P < 0.05 versus light-incubated samples.
FERMENTATION PRODUCTS IN MICROBIAL MATS 2349

A major fate of representative fermentation products in the Octopus Spring cyanobacterial mat is photoassimilation. Similar results were found for the fate of fermentation products in a high-sulfate hot spring cyanobacterial mat (39). Autoradiography revealed that these compounds are taken up by filamentous bacteria. As reported elsewhere (39), "'C-labeled filaments cross-reacted with Chloroflexus-specific antiserum (33). C. aurantiacus clearly plays an important role in controlling the fate of fermentation products in the light.

Fermentation products were metabolized to some extent in the dark under aerobic conditions (Table 1), although only ethanol and lactate were oxidized to "CO₂ in appreciable amounts. Under dark anaerobic conditions, and especially after extended preincubation under these conditions, there was little metabolism of acetate, propionate, and ethanol. Butyrate was assimilated (Table 1) and converted to acetate (Table 3), while lactate was rapidly oxidized to CO₂.

We investigated the potential for acetogenesis in hot spring mat samples which were incubated for several days under dark anaerobic conditions to select against photoheterotrophic processes and for acetogenesis. The catabolic

<table>
<thead>
<tr>
<th>Compound</th>
<th>Accumulation (μmol/μmol of acetate)</th>
<th>Ratio, 65°C/55°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>55°C</td>
<td>65°C</td>
</tr>
<tr>
<td>Propionate</td>
<td>0.310</td>
<td>0.074</td>
</tr>
<tr>
<td>n-Butyrate</td>
<td>0.029</td>
<td>0.039</td>
</tr>
<tr>
<td>iso-Butyrate</td>
<td>0.017</td>
<td>0.039</td>
</tr>
<tr>
<td>n-Valerate</td>
<td>0.028</td>
<td>0.025</td>
</tr>
<tr>
<td>iso-Valerate</td>
<td>0.025</td>
<td>0.069</td>
</tr>
<tr>
<td>Ethanol</td>
<td>0.004</td>
<td>0.076</td>
</tr>
</tbody>
</table>

*Normalized for the amount of acetate which accumulated in the same incubation period.

For each product, the normalized amount at 65°C is compared with the normalized amount at 55°C to show the increased (ratio >1) or decreased (ratio <1) importance of the product at the higher temperature.
reactions of obligate H⁺-reducing acetogenic bacteria require low H₂ concentrations. In nature acetogens depend on H₂ consumers, such as methanogenic or sulfate-reducing bacteria, to maintain a low H₂ level. The sensitivity of metabolism of [1-14C]butyrate to [14C]acetate to inhibition of methanogenesis (increase in H₂) suggested the involvement of acetogenic bacteria. We also succeeded in enriching butyrate-degrading acetogenic bacteria from the 50°C Octopus Spring mat. As mentioned above, there was little metabolism of [1-13C]propionate or [1-13C]ethanol under dark anaerobic conditions. The sensitivity of [1-13C]propionate conversion to 14CO₂ to inhibition of methanogenesis may suggest acetogenesis of this substrate as well, although no conversion of [1-13C]propionate to [14C]acetate was noted and we were unable to enrich propionate-degrading acetogenic bacteria from the mat.

Though only a few acetogenic bacteria are known (5, 23), thermophilic butyrate-degrading acetogenic bacteria have been isolated from a high-temperature waste digestor (12). Acetogenesis has been shown to occur in waste digesters acclimated to 55°C (11) and 60°C (20), although such digestors could not be operated efficiently at higher temperatures (34). The upper temperature limit for acetogenesis by the isolated thermophilic acetogen, or by natural populations in thermal habitats, has not been determined. However, as thermophilic representatives of the other major groups in anaerobic food chains, fermentative and methanogenic bacteria, prefer to grow above 60°C, it is possible that the thermal limitations of acetogenic bacteria may determine the upper temperature for efficient anaerobic decomposition.

As most fermentation products were not catabolized during dark anaerobic incubation of the Octopus Spring cyanobacterial mat, their accumulation should be a measure of their importance in carbon cycling. Acetate and propionate were the main products to accumulate in the Octopus Spring mat. Similar results were reported for the cyanobacterial mat in the high-sulfate hot spring Bath Lake (38). The predominance of acetate as a fermentation product has also been shown for many other anaerobic environments, including lake (17, 18), salt marsh (2) and marine sediments (32), and cattle waste digesters (20). Thus, fermentative carbon flow in hot spring mats appears to be typical of that in other anaerobic systems (37, 40).

The importance of acetate is probably due to interspecies hydrogen transfer between fermenting and hydrogen-consuming bacteria, which leads to decreased production of more reduced end products, such as lactate, ethanol, and other fatty acids. The comparison of product accumulation in 55 and 65°C mats provided a unique approach to evaluating the importance of hydrogen consumption on fermentation. The failure of terminal members of the anaerobic food chain (i.e., methanogens in Octopus Spring) to consume hydrogen leads to its accumulation at 65°C. More reduced products, notably ethanol, but also n- and iso-butyrate, iso-valerate, and lactate, accumulated during incubation of a 65°C mat than accumulated during incubation of a 55°C mat in which hydrogen was kept low by methanogenesis. Similarly, there was a greater relative increase in reduced fermentation products, such as ethanol, when methanogenesis in the 55°C mat was inhibited, causing accumulation of hydrogen.

Lactate was decomposed during dark anaerobic incubation of Octopus Spring mat samples (Tables 1 and 2), so that its importance as a fermentation product may be underestimated by following its accumulation. The difficulty in accurately determining the indigenous lactate concentration within the upper few millimeters of the mat prevented evaluation of the relative importance of this product in turnover studies.

How well the accumulation of butyrate reflects its importance as a product of fermentation is also unclear because of its metabolism under dark anaerobic conditions. The conversion of butyrate to acetate in the Octopus Spring cyanobacterial mat occurred during short incubations which were representative of natural conditions. No metabolism of butyrate to acetate was observed during light incubation. This could either be due to photoheterotrophic assimilation of butyrate by C.aurantiacus or to inhibition of acetogenesis, an anaerobic process, by photosynthetically derived O₂. Some conversion of butyrate to acetate was observed under dark aerobic conditions, but microelectrode studies suggest that mat samples incubated in this way are mostly anoxic despite the overlying oxygenated water (27). Comparison of the extent of butyrate conversion to acetate observed under dark aerobic and anaerobic conditions suggests that butyrate acetogenesis is sensitive to oxygen. The rapid conversion of butyrate to acetate observed under dark anaerobic conditions is particularly interesting, since it suggests that these anaerobes are able to recover from the superoxic conditions of sunlit mats (27) within 0.5 to 2.5 h of darkening and development of anaerobic conditions within the mat. Acetogenesis of butyrate is apparently not completely synchronized with fermentation since butyrate does accumulate during extended dark anaerobic incubation. In fact, the observed lower percent conversion of [1-14C]butyrate to [14C]acetate in samples preincubated anaerobically in darkness for 24 h before addition of radiolabel may be explained by an increase in butyrate concentration and, thus, a corresponding decrease in specific activity of the added radiolabel.

We were surprised to find that fermentation was as rapid at 65°C as at 50°C. Other anaerobic processes in hot spring mats are most active in the 50 to 55°C region of the mat (30, 36). We previously suspected that this was a result of the similar temperature distributions of photosynthetic mat formation and decomposition processes (27, 30), reasoning that decomposition might be limited by the supply of carbon (and energy) fixed by phototrophs. However, fermentation does not seem to be limited in this way. Apparently, terminal anaerobic groups are uncoupled from fermentation for other, as yet unknown, reasons.

Fermentation occurs mainly within the upper 4 mm of the mat. Similarly, methanogenesis (36) and sulfate reduction (38) were also found to be most active in the upper 5 mm of hot spring cyanobacterial mats. This positioning in a cyanobacterial mat presents problems for anaerobic microorganisms because of diurnal oxygen fluctuations caused by oxygenic photosynthesis. For example, the upper 2 to 10 mm of the mat is oxic during the day, and the upper 2 to 4 mm is typically superoxic; the upper regions of the mat are anoxic only at night (27). Light inhibition of the accumulation of fermentation products could be due to the assimilation of fermentation products by phototrophs and microorganisms in the photic zone, and is also likely due to oxygen inhibition of anaerobic processes. Anaerobes must either avoid or tolerate exposure to oxygen extremes. Some microbes reposition themselves by following moving vertical chemical gradients in mats (16, 24). Four of the fermentative isolates from Octopus Spring are motile, but it seems unlikely that these anaerobes reposition themselves to avoid oxygen exposure, as the penetration of oxygen in the sunlit mat is often as deep as 7 to 10 mm below the mat surface, and vertical profiles of fermentation
in mat samples collected at midday showed maximum fermentation (after a shift to dark anoxic conditions) within the top 4 mm. Some of the fermentative isolates from Octopus Spring are able to tolerate exposure to air, but all grow only under anaerobic conditions.

We hypothesize that fermentation and other anaerobic processes occur diurnally in these cyanobacterial mats. As some of the major products of fermentation are not degraded under dark anaerobic conditions, fermentation products should accumulate in the mat at night. This seems to be reflected in diurnal pH fluctuations which have been measured in the Octopus Spring mat (27). Between sunset and sunrise the pH of the top 5 mm of the mat decreases to about one pH unit less than that of the water overflowing the mat. The steep pH gradient at the mat surface at night implies export of fermentation products from the mat to the overlying water, which may represent loss of carbon and energy from the mat. Fermentation products are consumed by C. aurantiacus in the day, but continuous crossfeeding between fermenting and photoheterotrophic bacteria is unlikely, as high oxygen levels should inhibit fermentation during the day. In periods of low light intensity, when the lower portion of the photic zone may be anoxic, fermentation and photoheterotrophy may be concurrent. Phototrophic consumption of fermentation products should be most intense at sunrise, after a long period of accumulation of fermentation products at night. The natural population of fermentative microorganisms seems capable of responding within hours (i.e., shortly after the onset of darkness) to the development of anaerobic conditions, since fermentation products accumulated in samples collected in full sunlight and then incubated for a few hours in darkness.

The major differences in carbon cycling in hot spring cyanobacterial mats compared with other habitats appear to relate to the proximity of phototrophic and anaerobic decomposition processes, separated temporally (and by environmental conditions which vary with time of day) by more than distance. Carbon cycling may be similar in other aquatic communities which are spatially confined and exposed to light. Examples might include sediment communities of the littoral, subtidal, or intertidal zones and benthic algal mats. The interdependent fluctuations of light and oxygen are similar in both cyanobacterial mats (15, 27) and intertidal sediments (26). Anaerobic processes also show similar vertical distributions, being maximal near the surface of both mats (39) and sediments (40).

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LITERATURE CITED


