ANAEROBIC CHITIN DEGRADATION AS A CARBON AND HYDROGEN SOURCE FOR SULFATE REDUCTION AND METHANOGENESIS IN SALT MARSH BACTERIA

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Chitin is the major structural component of fungal cell walls and arthopod exoskeletons. Next to cellulose, it is probably the most abundant form of polymerized carbon in the marine system. Goodrich and Mortia (1977) estimated that a single planktonic crustecean, Euphausia pacifica, produces ten billion metric tons of molt chitin annually. Add to that all the micro-, meio-, and macrofaunal chitin associated with sediments and the amount of carbon tied up in chitin becomes considerable. Considering the abundance of chitin in nature, very little is known of its cycling in terms of rates, pathways, or products. What we tried to do is follow the anaerobic degradation of chitin to its end-products and to determine how these products are used by other sediment bacteria.

Figure 1 shows the known aerobic pathway of chitin mineralization by bacteria. Degradation proceeds through an inducible enzyme system to ammonia, carbon dioxide, and water. This is very similar to cellulose respiration. Under anoxic conditions, cellulose is broken down to volatile fatty acids, hydrogen, and carbon dioxide. Therefore, we postulated that chitin would be fermented via a parallel pathway. If this was true then we could expect similar products and also expect the scavenging of these products by sulfate reducers and methanogens as in detrital systems. To test this hypothesis, we had to isolate anaerobic chitin degraders from the marsh and analyze chitin breakdown products. We also wanted to isolate sulfate reducers and methanogens for subsequent growth studies on postulated products of chitin metabolism. In other words, the idea was to uncouple the marsh system for more detailed studies of its components.

METHODS

The media used for the selective isolation of salt marsh bacteria are listed in Table 1.

TABLE 1
MEDIA

	CHITIN DEGRADER	SULFATE REDUCER	METHANOGEN
Minimal Salts	+	+	+
Vitamins	+	+	+
Minerals	+	+	+
NaCl	+	+	+
Chitin	+		
Acetate		+	+
$FeSO_4$		+	
Na_2SO_4		+	
Yeast extract		+	+
Trypticase			+
Reducing Agent	+	+	+
Indicator	+	+	+
$N^2 - CO^2$	+	+	+

Chitin degrader medium consisted of minimal salts with particulate chitin as the sole carbon source. Sulfate reducer medium has acetate, hydrogen, and carbon dioxide as the carbon and energy sources along with minimal salts. Sodium sulfate and ferrous iron were incorporated into the medium to act as a sulfide indicator - when sulfate reduction occurs a dense black precipitate of FeS is formed. Methanogen medium consisted of acetate with hydrogen-carbon dioxide as carbon and reductant source. All media were prepared and dispenses in prereduced form using cysteine-sulfide of thioglycolate-ascorbic acid as reducing agent. Reazurin was incorporated into all media as an Eh indicator. If the Eh of the medium ever rose higher than -200mV, the tubes would turn pink.

All media manipulations were done in either Balch tubes or bottle plates. The Balch tube is a modified test tube with a serum bottle top that allows it to be sealed with a butyl rubber stopper and crimped in order to maintain an internal atmosphere. It is used primarily for broth cultures and slants. The bottle plate consists of a plain pharmaceutical bottle complete with rubber stopper and cap. Agar is poured into the bottle as it is lying horizontally so a thin layer is formed. This can then be streaked under nitrogen much as a petri dish. Both are maintained anoxically by the use of anaerobic gas manifolds.

Sediment samples were taken from the Great Sippiwissett marsh and kept under nitrogen during transfer. Samples were plated onto bottle plates of selective media using strict anaerobic Hungate technique. The formation of black colonies on acetate and hydrogen by the precipitation of FeS indicated the presence of exocellular chitinase. Headspace gas analysis by GC told us whether any isolates were producing methane. Transfers were made until pure cultures were obtained. Pure cultures of all three metabolic types were inoculated into chitin broth and assayed for growth after five days. Sulfate reducers and methanogens did not grow or produce any metabolic products from chitin. Isolates of chitin degraders grew well producing acetate, hydrogen, and carbon dioxide from chitin alone. A total of seventeen different colony types were noticed.

Since we knew that acetate, hydrogen, and carbon dioxide are products of cellulose fermentation, experiments were set up to see if cocultures of chitin degraders-sulfate reducers and chitin degraders-methanogens would produce sulfide and methane respectively. A final test was performed to see if methanogens could compete with sulfate reducers for the limiting resourse hydrogen.

RESULTS AND DISCUSSION

The data from one experiment using three marsh isolates are shown in Table 2.

Table 2
nmoles/100 nmoles chitin substrate

Products	C13	C13 & M2	C13 & SR3	C13 & M2 & SR3
Acetate	47.9	51.3	35.2	37.4
H_2	10.9	0	0	0
CO_2	0.7	0	0.1	0
CH_4	0	20.6	0	5.4
S_2	-	-	+	+

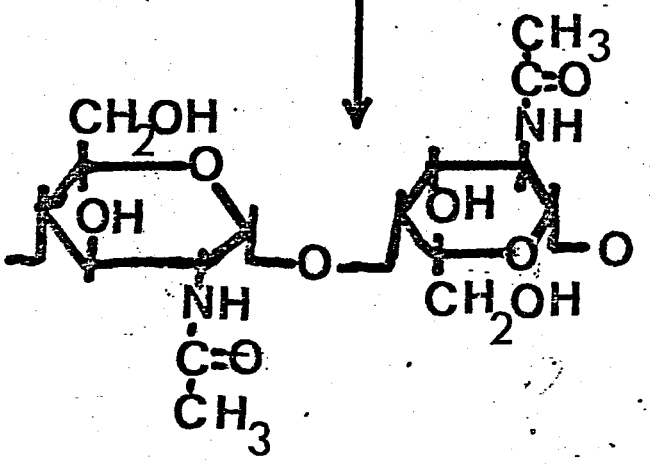
Other experiments were performed using different isolates but only a representative data set is shown. The chitin degrader produced acetate as previously mentioned. When a methanogenic isolate was added to a chitin degrader the hydrogen and carbon dioxide dropped out to form methane. The combination of chitin degrader and sulfate reducer also used hydrogen, most carbon dioxide, and blackened the tube indicating sulfide. Interestingly, the amount of acetate dropped by fifteen percent in tubes with sulfate reducers. This was probably due to the fact that some sulfate reducers can oxidize acetate to carbon dioxide while producing sulfide. In the last experiment, chitin degraders were combined with both sulfate reducers and methanogens to observe competition for hydrogen. We found reduced levels of acetate along with lower methane production in the presence of sulfate reduction. Clearly, there is interspecific competition for hydrogen in cocultures of sulfate reducers and methanogens grown on the breakdown products of chitin.

Figure 2 is the proposed pathway for anaerobic chitin degradation in salt marsh sediments. Chitin has been shown to ferment to acetate, hydrogen, and carbondioxide. These products have been shown to be scavenged by either sulfate reducers or methanogens under pure culture conditions. Methanogens have been found to compete for hydrogen normally destined for sulfate reduction. The occurrence of this competition in actual marsh sediments remains unresolved.

Figure 1

CHITIN POLYMER

Chitinase



Chitobiose

Chitobiase

N-acetylglucosamine

Deacetylase

Acetate

CO_2 H_2O

NH_4^+

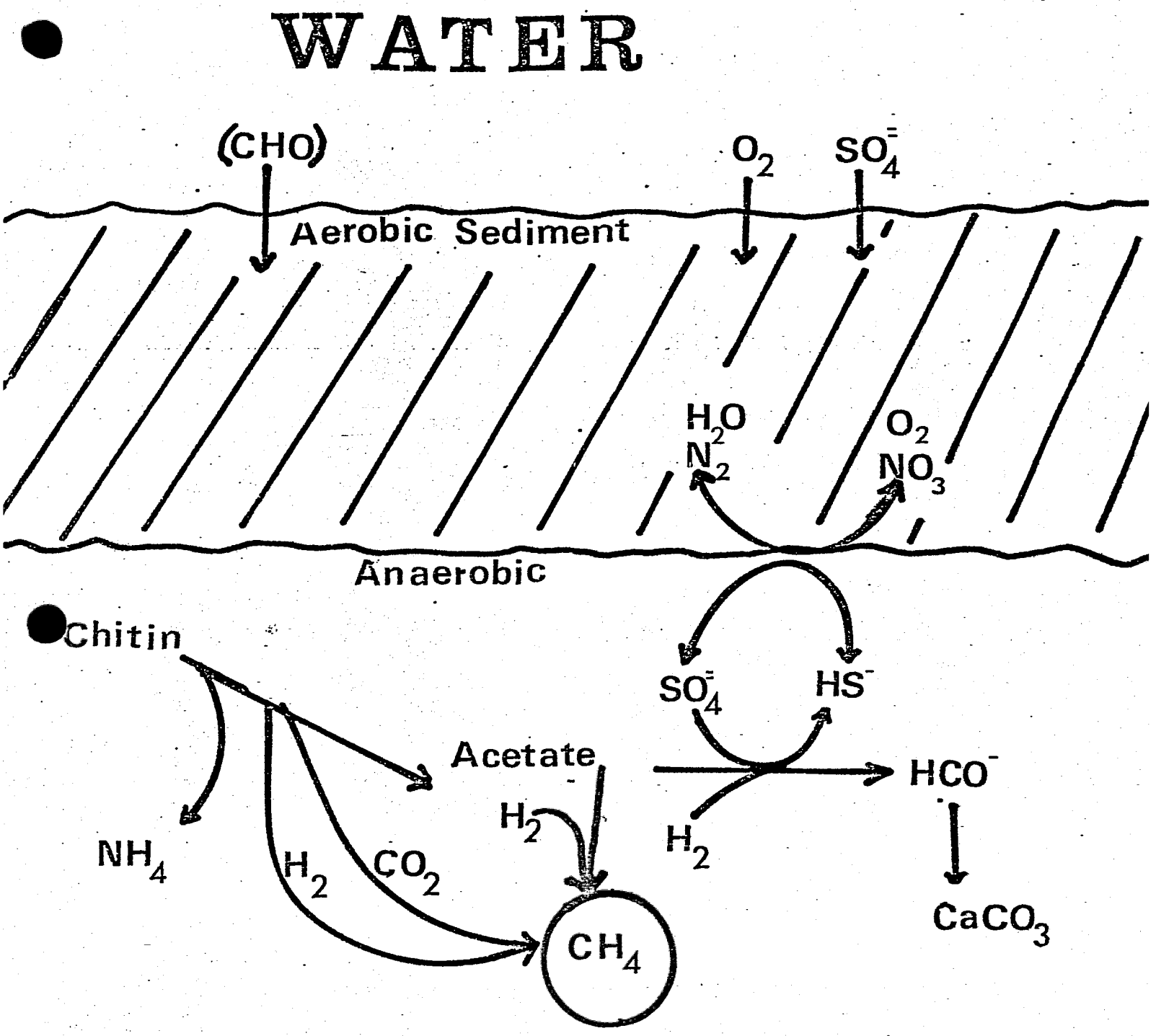
Glucosamine

Deaminase

Glucose

CO_2 + H_2O

Figure 2


WATER
(CHO)
O_2
$SO_4^=$
Aerobic Sediment
H_2O
N_2
O_2
NO_3
Anaerobic
Chitin
$SO_4^=$
HS^-
Acetate
HCO^-
H_2
H_2
NH_4
H_2
CO_2
CH_4
$CaCO_3$

FEEDING RELATIONSHIPS OF HARPACTICOID COPEPODS AND MICROBIAL FLORA IN A SALT MARSH

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INTRODUCTION

Benthic harpacticoid copepods represent a predonderate link in the estuarine food web. In the upper food web they serve as food for many invertebrates and fish fry. In the lower portion of the web, it has been suggested that they feed to a large extent on the microbial flora and are therefore important in the recycling of benthic energy. While their relationships with microbes have been the subject of much literature, information regarding the actual food resources of harpacticoids is limited. Most of these studies are strictly laboratory investigations, using food material which is either easily culturable or easily accessible but not necessarily representative of the animals' natural habitat.

In small drainage channels of Great Sippewisset Marsh there occurs definite zonation of photosynthetic microbial flora. The relationships of certain harpacticoid copepod species with these photosynthetic microbes (and their associated heterothrophic bacteria) are examined.

METHODS

Characterization of Organisms

The study site was in proximity to perennial microbial mats (Howarth and Marino, 1983). Across the surface sediment (0.05 cm depth) there is a distinct zonation of photosynthetic bacteria and diatoms, distinctly visible by the color patterns which they generate at high densities. The study site was divided into three areas, based on these zonations: 1) a "Diatom Area", located at the center of the channel, was composed of a dense mat of naviculoid diatoms; 2) a "Purple Area", located on either side of the diatom area, is composed of a photosynthetic purple-sulfur bacteria; 3) a "Clear Zone" dominated by neither the diatoms nor the purple-sulfur bacteria and located at the periphery of the channel.

Sampling was conducted during early July 1983 during low-water level. Two quadrats (20 x 15 cm) were placed in each area. Eight replicate cores (6 mm dia.) were randomly taken from each quadrat to a depth of 0.5 cm. Preliminary sampling indicated almost no animals below this depth. Cores were fixed on site in 10% formalin in seawater. Copepods were identified to species, densities recorded, then analyzed by ANOVA using BMDP computer programs.

For pigment analysis, frozen samples were thawed and the sediments filtered on Whatman GF/C glass fiber filters to remove excess water. Pigments were then extracted with 1 ml methanol (w $MgCo_3$) under darkness for 1 h and analyzed using a Gilford System 2600 Spectrophotometer from 660-800 nm. Within the diatom area, the photosynthetic organisms were composed almost exclusively of the naviculoid diatoms with lesser amounts of the cyanobacterium Oscillatoria sp. and underlain by purple sulfur bacteria. In the purple area the dominant photosynthetic organism was the purple-sulfur bacteria tentatively identified as Thiocapsa. Therefore pigment peaks of