Table 1. Incubation of slices from regenerating liver with N^{15} -glycine (excess N^{15} = 32 atom per cent) and N^{15} -orotic acid (excess N^{15} = 16.2 atom per cent). AO = ATP medium + 5 mg orotic acid; KO1 = Krebs-Henseleit medium + 5 mg orotic acid; KO2 = Krebs-Henseleit medium + 25 mg orotic acid; KG = Krebs-Henseleit medium + 50 mg glycine.

	Time of incubation	Excess N^{15}							
		PNA				DNA			
		Adenine	Guanine	Cytidine	Uridine	Adenine	Guanine	Thymine	Cytosine
AO	4 h			0.038	0.098			0.035	0.021
AO	8 h	0.008	0.006	0.102	0.281	0.007	0.003	0.096	0.073
KO1	4 h	0.017	0.009	0.057	0.140				
KO1	8 h	0.026	0.019	0.156	0.390	0.005	0.002	0.099	0.068
KO2	4 h	0.000	0.001	0.093	0.209				
KO2	8 h	0.015	0.011	0.227	0.504	0.006	0.009	0.160	0.139
KG	2 h	0.041	0.013						
KG	4 h	0.066	0.030	0.007	0.011				
KG	8 h	0.105	0.067	0.022	0.031	0.029	0.025	0.016	0.014

in mind that probably most of the isotope is located in only one nitrogen atom⁵ out of five.

Corresponding experiments with homogenates from regenerating liver in both types of media did not show a significant incorporation of N^{15} into the bases; apparently this system does not synthesize polynucleotides from these precursors.

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On Carbon Balance and Carbon Dioxide Fixation in Thermophilic Cellulose Fermentation

L. ENEBO

Division of Microbiology, Royal Institute of Technology, Stockholm, Sweden

In experiments with six different pure cultures of thermophilic bacteria on the carbon balance in fermentation of cellulose McBee¹ was able to obtain a satisfactory recovery of the carbon from the fermented cellulose in only one case. Similarly Imsenecki² in his experiments with a possibly pure culture* obtained a recovery of only c. 75 % of the carbon added. It should be noticed, however, that both these authors neglected succinic acid which appeared only as a trace among the products.

It seems probable that small changes in the general condition of the fermentation

* In spite of the doubtful isolation method it seems with regard to more recent results of McBee^{1,3} and the author^{4,5} that the culture of Imsenecki really was pure.

have a rather important influence on the composition of the products. Thus the author has obtained a better carbon recovery when working with more concentrated cellulose suspensions than McBee. The culture was isolated from the same mixed stock culture from which McBee isolated his strain EB¹.

The basal medium was a suspension of 10 g/l cellulose wadding and 10 g/l CaCO₃ in a solution of the following composition:

(NH ₄) ₂ SO ₄	2 g/l
KH ₂ PO ₄	1 »
MgSO ₄ · 7 H ₂ O	0.5 »
NaCl	0.5 »
Biotin	2 µg/l

In experiments A also 2 g/l Bacto tryptone (Difco) and 2 g/l yeast autolyzate of bakers' yeast were added.

The fermentations were carried out anaerobically with 0.5 l medium in all

Table 1. Experiments A. Products obtained in fermentation of cellulose with a pure culture of cellulose thermophiles. Medium containing tryptone and yeast autolyzate. Incubation for 10 days at 55° C.

products per g of cellulose consumed:

Experiment no.	1	2	3	4	5	6	7
	g mM	g mM	g mM	g mM	g mM	g mM	g mM
Products of fermentation:							
Carbon dioxide	0.072 1.64	0.072 1.64	0.072 1.64	0.072 1.64	0.065 1.48	0.069 1.57	0.081 1.84
Ethanol	0.056 1.22	0.056 1.22	0.044 0.96	0.051 1.11	0.040 0.87	0.039 0.85	0.048 1.04
Formic acid	0.017 0.37	0.041 0.89	0.015 0.33	0.014 0.30	0.022 0.48	0.017 0.37	0.013 0.28
Acetic acid	0.160 2.67	0.069 1.15	0.151 2.52	0.106 1.77	0.169 2.82	0.125 2.08	0.103 1.72
Lactic acid	0.253 2.81	0.160 1.78	0.145 1.61	0.252 2.80	0.304 3.38	0.151 1.68	0.289 3.21
Succinic acid	0.055 0.47	0.140 1.19	0.113 0.96	0.098 0.83	0.132 1.12	0.128 1.09	0.060 0.51
Glucose	0.437 2.43	0.576 3.20	0.565 3.14	0.473 2.63	0.408 2.27	0.586 3.26	0.509 2.83

Table 1 a. Carbon recovery in experiments A.

g carbon obtained per g of cellulose consumed:

Experiment no.	1	2	3	4	5	6	7
Products of fermentation:							
Carbon dioxide	0.020	0.020	0.020	0.020	0.018	0.019	0.022
Ethanol	0.030	0.030	0.023	0.027	0.021	0.020	0.025
Formic acid	0.005	0.011	0.004	0.004	0.006	0.005	0.004
Acetic acid	0.067	0.028	0.060	0.042	0.068	0.050	0.041
Lactic acid	0.101	0.064	0.058	0.101	0.123	0.060	0.116
Succinic acid	0.024	0.057	0.046	0.040	0.054	0.052	0.024
Glucose	0.186	0.230	0.226	0.189	0.163	0.234	0.203
Total carbon recovery, g	0.433	0.440	0.437	0.423	0.453	0.440	0.435
Total carbon recovery in per cent of theoretical value	97.3	98.9	98.2	95.1	101.8	98.9	97.8

Table 2. *Experiments B. Results of fermentation of cellulose in an inorganic salt solution with a pure culture of cellulose thermophiles. Incubation for 10 days at 55° C.*

Experiment no.	1			2			3		
	Ferm. pro- ducts per g cellulose consumed	g C per g cellulose consumed		Ferm. pro- ducts per g cellulose consumed	g C per g cellulose consumed		Ferm. pro- ducts per g cellulose consumed	g C per g cellulose consumed	
	Mm	g		Mm	g		Mm	g	
Products of fermentation:									
Hydrogen	5.45	0.0109	—	6.35	0.0127	—	6.70	0.0134	—
Carbon dioxide	3.39	0.149	0.041	3.64	0.160	0.044	3.98	0.175	0.048
Ethanol	1.00	0.046	0.024	1.13	0.052	0.027	0.93	0.043	0.022
Formic acid	0.26	0.012	0.003	0.33	0.015	0.004	0.30	0.014	0.004
Acetic acid	3.28	0.197	0.079	3.22	0.193	0.077	3.07	0.184	0.074
Lactic acid	2.71	0.244	0.098	1.92	0.173	0.069	1.60	0.144	0.058
Succinic acid	0.71	0.084	0.034	1.14	0.135	0.055	0.97	0.114	0.046
Glucose	2.31	0.416	0.166	2.46	0.442	0.177	2.72	0.490	0.196
Total carbon recovery, g			0.445			0.453			0.448
Total carbon recovery in per cent of theoretical value			100.0			101.8			100.7
Oxidation-re- duction index		1.04			1.02			1.08	

glass apparatus. As reducing agent 0.03 per cent thioglycollic acid was added.

In both cases a satisfactory recovery was obtained. It is probable that tryptone and yeast autolyzate in experiments A to a small extent have served as a source of carbon, but experiments B clearly show that the carbon recovery can also be as high in the absence of these organic nutrients.

The maximum influence of the thioglycollic acid on the oxidation-reduction index in the present case would have been c. + 0.03 if oxidation had been complete during the fermentation. This, however, was not the case.

The carbon recovery is very high, in spite of the fact that the carbon content of the bacterial cells formed during the

fermentation was omitted in the calculation. A certain utilization of CO₂ for the synthesis of other products may therefore be assumed. A preliminary attempt to demonstrate CO₂-fixation was carried out.

Fermentations were made with 100 ml each of the medium mentioned above, but omitting tryptone and yeast autolyzate, and with addition of a small amount of CaC¹⁴O₃ to the buffer.

Table 3 shows that the CO₂-fixation is very minute and possibly limited only to the formation of succinic acid. 1.15 per cent of the carbon dioxide added has taken part in the formation of fermentation products. Calculated on basis of carbon in the products and assuming 1.15 per cent of fixed carbon dioxide, the influence of CO₂-fixation in the carbon balance is about

Table 3. Results of a pure culture thermophilic cellulose fermentation with addition of $\text{CaC}^{14}\text{O}_3$.

Products	mg	mg carbon	counts per mg and minute	total counts per minute
Ethanol	61	32	23	1.400
Formic acid	10	3	81	810
Acetic acid	87	35	4.5	390
Lactic acid	67	27	88	5.900
Succinic acid	69	28	808	56.000
Total	294	125		64.500
Carbon dioxide, added	102	38	54.700	5.580.000
Cellulose, consumed	473	210		

2 pro mille. The succinic acid formed by means of CO_2 -fixation corresponds to 1.6 per cent of the total succinic acid formation. Experiments are being planned in order to establish if more CO_2 would be fixed when the fermentation is carried out under higher CO_2 -pressure than in the present case.

It should be noticed that Virtanen⁶ and Koistinen⁷ have demonstrated CO_2 -fixation in cellulose fermentation with mixed cultures, but it was not proved that the fixation was not performed by the symbionts.

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Chemistry of the Histochemical Phosphatase Reaction

H. HOLTER, S. LØVTRUP and INGER RUBIN

Cytochemical Department, Carlsberg Laboratory, Copenhagen, Denmark

Gomori's² and Takamatsu's³ reaction for the histochemical detection of phosphatase in tissues is based on the following principle: The tissue is fixed in alcohol or acetone, dehydrated, embedded in paraffin and sectioned on the microtome. The deparaffinized sections are then incubated at $\text{pH} > 9$ with the phosphate ester (usually glycerophosphate) in the presence of calcium ions. Upon liberation of inorganic phosphate by enzymatic cleavage a precipitate of hydroxy-apatite is formed. Then the tissue is treated with cobalt nitrate in order to convert the precipitate to cobalt phosphate, and finally with ammonium sulfide in order to convert the cobalt phosphate into cobalt sulfide. The latter precipitate is black and reveals upon inspection in the microscope the presumed sites of phosphatase activity.

The technique is widely used and various variants of the method have been developed employing other combinations of substrates, salts and pH. In all of these, however, the principle is the same, since a colourless product of the enzymatic reaction is by suitable treatment converted into a colored substance which can be observed in the microscope. A number of critical investigations of the technique have been carried out by various authors, in order to clear up certain points which are vital for its validity, such as inactivation of the enzyme during the preliminary histological treatment, secondary effects of diffusion and re-adsorption, etc. As far as we are aware, however, the question of the conversion of the initial precipitate into the stained end-product has not received similar attention. In order to test this