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BRIEF COMMUNICATIONS

Bioconversion of D-Glucose in Heavy Water: Effect of Water Isotopomeric Composition on Deuterium Fragment Distribution in Ethanol

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Abstract—The relative distribution of deuterium between methyl and methylene groups in ethanol at the bioconversion of D-glucose in heavy water was studied.

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It is known that hydrogen atoms in heavy water are redistributed to form three isotopomeric forms: HOH, HO^2H and $^2HO^2H$, the ratio between which depending on a deuterium content in it [1]. On the assumption that such equilibrium can influence the direction of hydrogen exchange in an organic reaction, we have studied in this work the fragment distribution of deuterium in ethanol obtained on the bioconversion of D-glucose in heavy water, varying deuterium concentration in it from 5 up to 99%. The isotopomeric composition of the resulting product, which is presented in the table, was determined by the method of quantitative ^{13}C NMR spectroscopy [2].

Examination of the presented data shows that alcohol molecules monodeuterated at the methyl or methylene group are mainly formed in the isotope-dilute medium in the ratio close to 1 : 2, respectively. As the concentration of heavy water increases, the spectrum of the formed isotopomers essentially extends up to the complete substitution of deuterium (3.2%) for protium in the ethyl group. Correspondingly the deuterium contents in the

Effect of heavy water concentration on the deuterium fragment distribution in ethanol on the D-glucose fermentation in the presence of *Saccharomyces cerevisiae*

Heavy water concentration, %	Isotopomeric composition, %											D_I^a	D_{II}^a	D_I^a/D_{II}^a
	$[^2H_0]$–	$[1\text{-}^2H_1]$–	$[1\text{-}^2H_2]$–	$[2\text{-}^2H_1]$–	$[1,2\text{-}^2H_2]$–	$[1,1,2\text{-}^2H_3]$–	$[2\text{-}^2H_2]$–	$[1,2,2\text{-}^2H_3]$–	$[1,1,2,2\text{-}^2H_4]$–	$[1,2,2,2\text{-}^2H_4]$–	$[1,2\text{-}^2H_5]$–	g-atom		
5	91.6	5.8	–	2.6	–	–	–	–	–	–	–	0.058	0.026	2.23
10	83.6	10.4	0.4	4.9	0.7	–	–	–	–	–	–	0.119	0.056	2.13
25	58.5	23.7	2.4	9.3	4.0	0.6	0.7	0.5	0.2	–	–	0.346	0.167	2.07
50	29.3	30.1	7.7	11.6	12.8	3.8	1.9	2.1	0.7	–	–	0.694	0.376	1.85
75	8.5	21.9	12.8	8.2	20.2	13.1	3.1	7.4	4.8	–	–	1.109	0.721	1.54
99	1.3	7.7	11.5	2.6	15.6	25.3	2.0	11.1	17.7	2.0	3.2	1.518	1.207	1.26

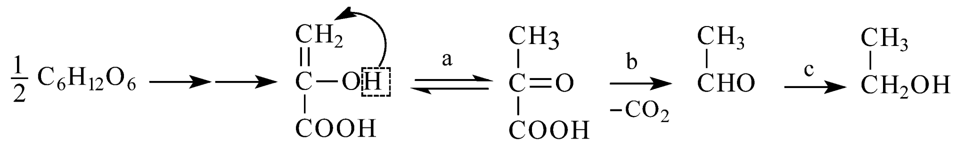
[a] D_I and D_{II} are deuterium contents in the methylene and methyl groups of alcohol.

methylene and methyl groups grow, this growth being noticeably faster in the latter, therefore differences in the relative isotope content in it and in the methylene group are gradually leveled, decreasing from 2.23 in 5% heavy water to 1.26 in concentrated heavy water (99%). The deuterium distribution in ethanol for each experiment was compared with the isotopomeric composition of water in use, as calculated by the binomial distribution equation [3].

Thus, it has been found that in the case of the D-glucose bioconversion in deuterowater solutions the observed leveling of differences in relative deuterium contents in methyl and methylene groups of ethanol coincides with the appearance of significant amounts of the d_2 isotopomeric form in water and manifests itself the more strongly, the higher is its content, reaching a maximal value in concentrated heavy water. Moreover, it has been found that the distribution curves of heavy water isotopomers d_0, d_1, and d_2 are reproduced practically completely also for ethanol, d_0, d_1, and d_{2-5}, respectively (Fig. 1). A negligible deviation is connected with the fact that, as a heavy water concentration increases, the fraction of the alcohol molecules [1,2-$^2H_2$]-CH_3CH_2OH, which are the d_1 isotopomer by fragments and d_2, by the gross-content, grows. As they are referred completely to the isotope group d_{2-5}, the content of the first isotopomers is lower and of the second higher compared to the corresponding isotopomers in water. It is evident that the observed analogy in the distribution of isotopomers in water and in alcohol formed in it is connected with a special feature of exchange reactions inside the substrate-enzyme complex.

The general view of the ^{13}C NMR spectrum of the methyl group of ethanol obtained by the glucose fragmentation in 99% heavy water is presented in Fig. 2.

The interpretation of the results of the present work was fulfilled within the limits of the Embden–Mayerhoff–Parnas standard scheme, according to which hydrogen atoms of water inside the substrate-enzyme complex can be brought into fructose as a result of the isotope substitution at the C_2 carbon atom [4] or in final stages of the conversion of enolpyruvic acid to ethanol (reactions a, b, and c) [5]:



$$\frac{1}{2}C_6H_{12}O_6 \longrightarrow\longrightarrow \begin{array}{c} CH_2 \\ \| \\ C{-}O\boxed{H} \\ | \\ COOH \end{array} \xrightleftharpoons{a} \begin{array}{c} CH_3 \\ | \\ C{=}O \\ | \\ COOH \end{array} \xrightarrow[-CO_2]{b} \begin{array}{c} CH_3 \\ | \\ CHO \end{array} \xrightarrow{c} \begin{array}{c} CH_3 \\ | \\ CH_2OH \end{array}$$

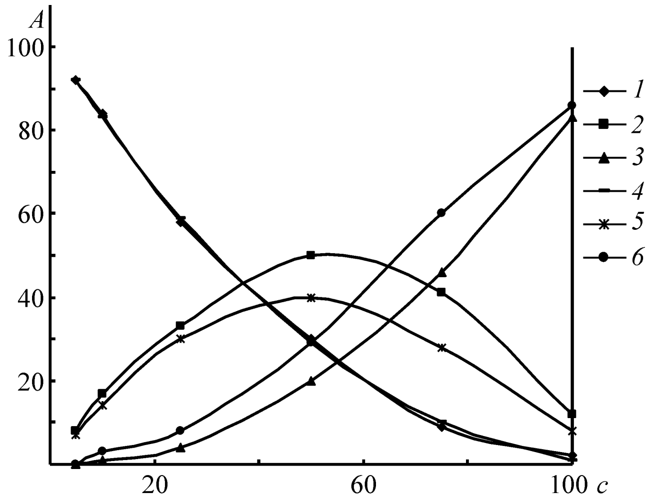


Fig. 1. Dependence of the contents *A* (%) of isotopomeric water forms and ethanol on the concentration *c* (%) of ^2H_2O in the medium. (*1*) HOH, (*2*) HO^2H, (*3*) $^2HO^2H$, (*4*) d_0-EtOH, (*5*) d_1-EtOH, (*6*) d_2-d_5-EtOH.

As deuterium is present in isotope-dilute water in the form of HO^2H molecules, it is obvious that the intermolecular interchanging in the hexose stage occurs preferentially due to the protium atom because of the isotopic effect. Thus, under these conditions the deuterium atom of water participates in the formation of the ethanol molecule mainly in the second stage as a result of enolpyruvic acid conversions. This conclusion is supported by the fragment ratio of deuterium in the methylene and methyl groups, (2.23–2.07) : 1, respectively, that practically corresponds to the number of vacant places at the molecule kitting-up from enolpyruvic acid up to ethanol: two for the methylene group and one for the methyl group.

When the heavy water concentration is increased the isotopic equilibrium in it is displaced aside the d_2 isotopomers. In their presence the possibility of the isotope alternation in the first stage is limited, therefore deuterium atoms are preferentially involved in the intermolecular exchange of hexoses, thereby providing its additional entering in the methyl group of ethanol proportionally to the content of these molecules in water. Thus, the total content of deuterium in the methyl group starts to grow noticeably faster than in the methylene group.

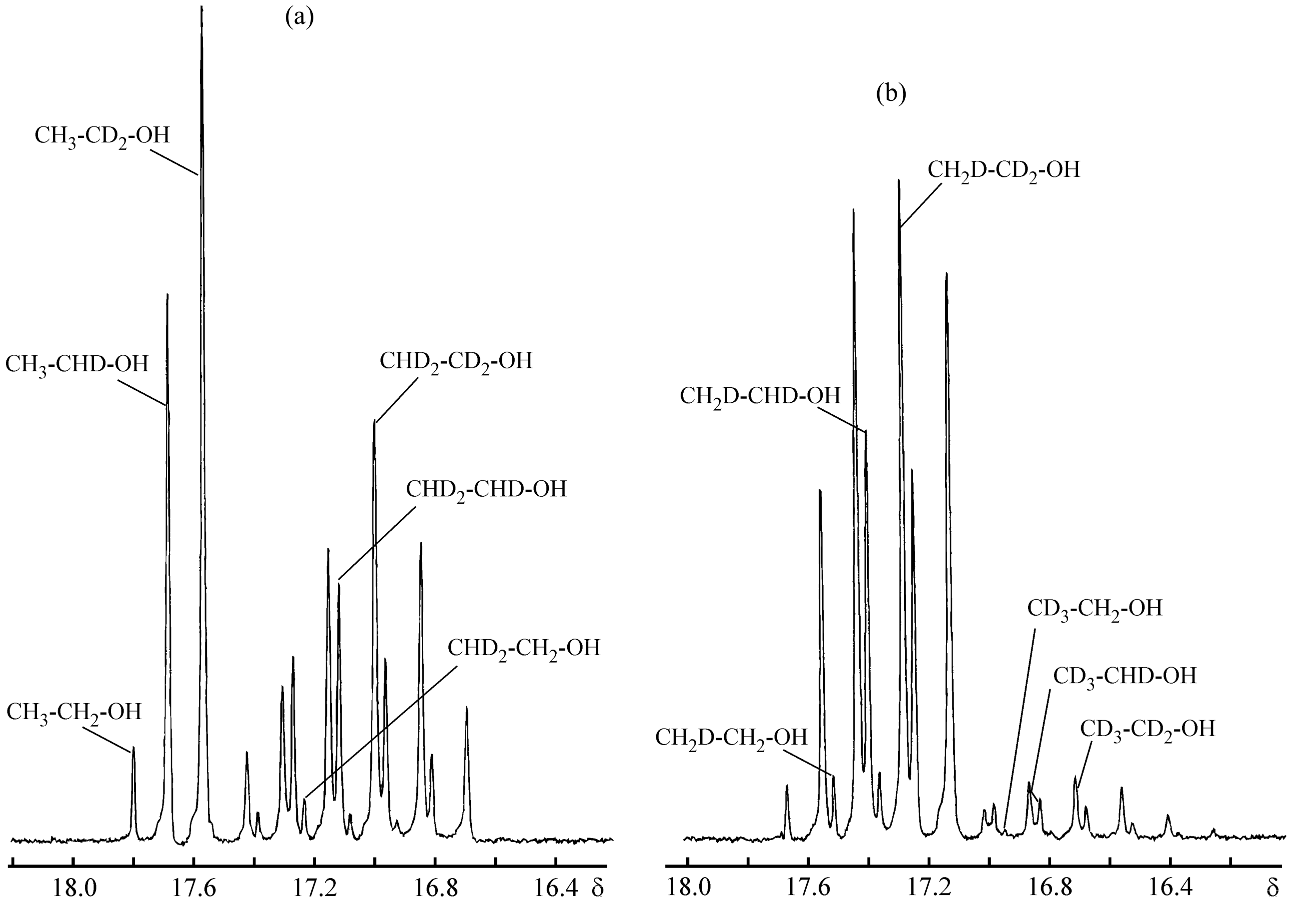


Fig. 2. Edited ^{13}C NMR spectra of isotopomers of ethanol methyl group obtained by the spin-echo technique. (δ) chemical shift (ppm). Isotopomer: (a) d_0, d_2; (b) d_1, d_3 (central components of multiples are specified).

EXPERIMENTAL

We used pure-grade commercial D-glucose in the experiments on the enzymatic fermentation. The aqueous medium with various deuterium contents was prepared by dilution of "Merck" heavy water. The isotope concentration of the resulting solutions was monitored by the ^2H NMR method. As ferment we applied baker's yeast grown up from the strain of *Saccharomyces cerevisiae* microorganism (cipher L80U).

A mixture of glucose (10 g), yeast (1.5 g), and water (30 ml) was held at room temperature within 240 h with periodical intensive stirring. After centrifugation and repeated distillation the yield of alcohol was 75%.

The NMR spectra were recorded on a VXR-500S Varian spectrometer, using precision Aldrich ampoules of 5 mm in diameter.

The isotopomeric composition of samples was calculated on the basis of the quantitative ^{13}C NMR spectra (resonance frequency of 125.7 MHz) recorded under proton irradiation. The width of the spectral sweep was 20 kHz after 256 impulses. Edited spectra of isotopomers were obtained by the spin-echo technique [modified program APT (Attached Proton Test)]. In all experiments the irradiation of protons was switched off during a relaxation delay, which lasted 20 s, and the pulse duration was 90°. The effect of the long-time instability of the instrumentation was eliminated by the application of multiple alternation at the accumulation of spin-echo subspectra participating in the editing. Resonance conditions were stabilized directly by the signal of deuterated samples.

CONCLUSIONS

(1) A heavy water concentration affects the deuterium fragment distribution at the bioconversion of D-glucose in the presence of *Saccharomyces cerevisiae*.

(2) It was found that the relative content of deuterium in methyl and methylene groups of ethanol depends on the isotopomeric composition of initial water.

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