Assimilation Spectrum of the Yeast *Candida utilis* 49 Used for Producing Fodder Yeast from Synthetic Ethanol

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**ABSTRACT.** Oxidizing and assimilating ability of the yeast *Candida utilis* 49 was tested with 21 different low-boiling organic compounds which come as components of raw synthetic ethanol. The highest yields of yeast dry weight were obtained with ethanol (72.0%), propanol (48.2%), ethyl acetate (43.4%) and acetic acid (34.2%). To a minor extent, the yeast was capable of utilizing also 2-propanol, butanol and 2-butanol; it oxidized most of the compounds tested.

The rapid development of utilization of non-sugar and synthetic substrates for the production of microbial single cell protein (SCP) in the last five years has been reflected in a considerable number of studies of methanol metabolism in yeast and bacterial strains newly isolated from naturally occurring sources (McLennan *et al.*, 1973; van Dijken and Harder, 1974; Mimura *et al.*, 1974; Oki and Kitai, 1974; Reuss *et al.*, 1974; Snedecor and Cooney, 1974; Blagodatskaya *et al.*, 1975; Sahm *et al.*, 1975; Tezuka *et al.*, 1975; Volfóva, 1975; Wagner and Levitch, 1975).

Many studies deal also with the utilization of ethanol, and several large research and industrial establishments are pursuing the industrial production of SCP from synthetic ethanol using yeasts of various genera (Kharat’yan *et al.*, 1974, 1975; Guiraud *et al.*, 1974; Masuda, 1974; Masuda *et al.*, 1974; Amano *et al.*, 1975a,b).

Relatively little attention was given to the microbial utilization of higher alcohols (Jackson, 1973; Mates, 1974; Matsumoto and Sato, 1974; Murooka and Harada, 1974), lower fatty acids (Huňková, 1972; Kinsel and Leathen, 1973; Sustina *et al.*, 1973; Dijkhuizen and Harder, 1975; Wadzinski and Ribbons, 1975) and carbonyl compounds (McGucken and Woodside, 1973; Ibragimova and Sakharova, 1974; Thomas and Russel, 1975; Veselov *et al.*, 1974).

Synthetic ethanol produced by ethylene hydration can contain, according to the conditions of synthesis and product refining, various organic compounds belonging to lower alcohols, ethers, aldehydes, ketones, etc. Literary data on the effect of these impurities on the production of SCP are scarce (Uher, 1973).

The present work was devoted to the investigation of the oxidation and assimilation of low-boiling organic compounds found as impurities in raw synthetic ethanol. We endeavoured to determine the oxidation and assimilation spectrum of the strain *Candida utilis* 49 which is being contemplated as a suitable strain for industrial production of SCP from synthetic ethanol.
MATERIALS AND METHODS

Microorganism and preparation of inoculum suspension. The yeast Candida utilis from the collection of our institute, was maintained on malt agar slopes and transferred monthly. Inoculum suspension was prepared by twice repeated 24-h cultivation in 500-ml boiling flasks containing 100 ml sterilized (20 min at 111590 Pa) medium containing 4.8 g (NH₄)₂SO₄, 0.65 g KH₂PO₄, 0.25 g MgSO₄·7 H₂O, 0.01 g ZnSO₄·7 H₂O, and tap water up to 1000 ml. The pH of the medium was 5.0. For the first cultivation the flasks were inoculated with 48-h-old colonies grown on agar slopes in a test tube, the inoculum for the second cultivation was one tenth of the volume of cultivation fluid from the terminated first cultivation.

After the second cultivation the cells were washed twice, centrifuged, and resuspended in 0.02M phosphate buffer (for oxidation tests) or in physiological saline (for assimilation tests).

Cultivation procedure and composition of media. Oxidation tests were carried out in 50-ml test tubes closed by ground glass stoppers, containing 10 ml yeast suspension (10 mg yeast dry weight/ml) in 0.02M phosphate buffer (pH 6.1) and 10 ml 1% (w/v) aqueous solution of the tested compound. The compounds under study included: methanol, acetaldehyde, diethyl ether, hexane, ethanol, 2-propanol, propanol, acetone, methyl ethyl ketone, ethyl acetate, 2-methylpropane-2-ol, 2-butanol, 2-methylpropane-1-ol, butanol, butyraldehyde, crotyl alcohol, crotonaldehyde, allyl alcohol, acrolein, acetic acid, and crotonic acid. The test tubes were incubated for 20–24 h in a thermostat at 30 °C, their contents filtered and the filtrates were used for the determination of acidity, content of residual substrate, and the formation of volatile product (by gas chromatography).

Assimilation of the compounds tested was done in a medium containing 2.18 g urea, 0.65 g KH₂PO₄, 0.25 g MgSO₄·7 H₂O, 0.01 g ZnSO₄·7 H₂O and tap water ad 1000 ml, with pH 5.5. The medium was supplemented with 1% (w/v) solution of the compound in question and inoculated with 2 ml yeast suspension (100 mg yeast dry weight/ml). The total volume of cultivation fluid in 500-ml flasks was 104 ml. The flasks were cultivated for 20–24 h at 30 °C on a rotary shaker (oxygen transfer 100 mmol O₂ 1⁻¹ h⁻¹) and the contents were analyzed for pH, biomass yield, formation of products and residual substrate.

Chemicals. Refined hydrogenated ethanol (East-Bohemian Distilleries, Chrudim) was purified of carbonyl substances by boiling with 2,4-dinitrophenylhydrazine and by distillation. Crotonaldehyde, acrolein, allyl alcohol and butyraldehyde (obtained from the Faculty of Organic Chemistry and Technology, Institute of Technology, Prague) were purified by distillation; crotonic acid was purified by crystallization. All other chemicals, mostly of reagent grade purity (Lachema, Brno), were distilled.

Analytical methods. Dry weight. 10 ml yeast suspension was filtered in an S₄ filter crucible, the cells were washed with distilled water and dried to constant weight at 105 °C.

Total nitrogen was determined by Kjeldahl micromethod.

Ammonia nitrogen was determined by distilling 10 ml filtered medium with the addition of MgO and titrating the distillate with sulphuric acid with an indicator according to Ma and Zuazaga.

The level of residual substrates and products was assayed in filtrates or their distillates by gas chromatography on a modified Chrom II apparatus with flame-ionization detection (Laboratorní přístroje, Prague). The conditions were: glass column (3.0 m . 3 mm) filled with Porapak Q (80–100 mesh), column temperature 182 °C, injector chamber temperature 220 °C. Carrier gas flow rate: 26 ml N₂/min; 35 ml H₂/min;