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## Intensification of higher alcohols biosynthesis – an advanced feedstock for biofuel production

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### Abstract

At present the problem of alternatives to fossil fuel is so critical that it sets an objective to search optimal renewable feedstock for biofuel. Being an alcohol production by-product fusel oil consists of higher volatile alcohols and can become such feedstock. Fusel oil is theoretically possible to process by the alcohol to jet (AtJ) method converting it into biojet fuel. Thus, it is reasonable to intensify the higher alcohols biosynthesis to increase the efficiency of biofuel production. However, it tends to be problematic to reach higher than average 0.35 % of ethanol yield of higher alcohols within industrial conditions. This paper shows the increase of up to 0.82 % of ethanol under modeled industrial conditions. Also the theoretical maximum of achievable increasing of up to 3.5 % of ethanol has been calculated.

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*Keywords:* fusel oil; higher alcohols; biofuel; intensification

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### 1. Introduction

The interest to biofuels has increased through the last years. One of the most important questions in biofuel production is the searching of potential renewable feedstock. The feedstock ranges from different wastes, macro- and microalgae to plant oils, potato, beef tallow and tobacco [1–5]. It has been estimated that these types of feedstock tend

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to decrease CO<sub>2</sub> emissions from transport [6]. One of such feedstocks can be higher volatile alcohols (HVA) producing in ethanol fermentation of carbohydrate substrates by yeast. Higher alcohols are separated from ethanol during rectification and traditionally named "fusel oil". Fusel oil essentially contains isobutyl and isoamyl alcohols in ratio 1:4. Fusel oil yields produced during fermentation can range from 0.1 to 1.1 % (average 0.35 %) of ethanol depending on the processing feedstock and fermentation conditions [7–9]. With the annual worldwide fuel ethanol production of 97.2 billion liters (in 2015 [10]) the amount of fusel oil produced can be estimated as 340 million liters per year.

Fusel oils are currently treated as by-product and are not used efficiently. Therefore, methods of utilization by adding fusel oil to fossil fuel [7–9, 11] or its converting into biodiesel [12, 13] are developing. There are also ways of converting alcohols into hydrocarbon fuels [14, 15]. One of these methods is AtJ (Alcohol to Jet) the 3-stage processing including such operations as dehydration, oligomerization and hydrogenation [15].

Higher alcohols formation is considered to occur with amino acids assimilation by Ehrlich pathway in anaerobic process of alcohol fermentation. By this pathway assimilation of valine results in isobutanol formation, leucine – 3-methylbutanol-1, isoleucine – 2-methylbutanol-1 (isoamyl alcohol isomers) [16, 17].

Taking into account the interest to fusel oil the awareness of its biosynthesis regulation can allow to change technological parameters of fermentation to intensify higher alcohols yield with their further use in biofuel production.

## 2. Materials and Methods

### 2.1. Yeast strains & Medium

The industrial culture *S. cerevisiae* Y-2396 was obtained from Russian National Collection of Industrial Microorganisms (VKPM).

A wort of 18 % dry matter obtained by saccharifying of semolina was used as substrate for fermentation. Enzymatic hydrolysis of semolina starch was carried out under traditional industrial regime in two stages. Thermostable  $\alpha$ -amylase was used at the first stage (pH 6.5, T = 90 °C, dosage 0.25 ml/kg of starch) and glucoamylase at the second one (pH 5.0, T = 60 °C, dosage 0.8 ml/kg of starch).

### 2.2. Analytical methods

The concentrations of isobutanol and isoamyl alcohol were determined by means of gas-liquid chromatography. The measurements were carried out in sample distillates using HP-4890 chromatograph by Hewlett-Packard packed with FFAP column 50 x 0.32 x 0.25. Helium flow rate was 7.0 ml/min. The oven temperature was set to isothermal regime of 200 °C. The injector temperature was 220 °C.

The inoculate concentration used was 5 g/l in all cases. The fermentation temperature was 30 °C except for the study of fermentation temperature effect.

## 3. Results and discussion

### 3.1. Formation of higher alcohols with the use of diverse nitrogen sources

Since HVA are considered to be formed from amino acids the experiment in addition of corresponding amino acids (leucine and valine) to the fermentation media was carried out. Also, the effect of addition of ammonia nitrogen (in the form of ammonium sulphate (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>) was studied in the experiment.

Table 1 shows the results of fermentation with diverse concentration of amino acids and ammonia nitrogen.

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