

## Composition and properties of basic high molecular weight impurities in waste waters from ethanol and fodder yeast production from wood biomass processing

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Analysis of the composition and properties of impurities of waste waters from ethanol and fodder yeast production shows that about 76% of impurities are retained in the ethanol-soluble fraction. Colloidal waste impurity particles average about 670 nm diameter.

When designing water recycling schemes for ethanol and fodder yeast production by wood biomass hydrolytic and biotechnological processing, particular attention should be given to the recycled waters quality and accumulation of various substances which inhibit chemical and biochemical processes in the main production. One of the basic problems in using waste to provide suitable recycling water quality and further, the stable functioning of the recycling system, is the presence of high molecular weight impurities with various degree of dispersion, which can not be biologically oxidized by local or extra-system purification. Pollution of the flow is caused by pentose and hexose sugars, organic acids, furfural, organic and inorganic compounds of nitrogen, lignin, and lignohumic substances (LHS) (products of polycondensation of phenol compounds formed by thermocatalytic destruction of lignin in acidic media, furfural and oximethylfurfural, and products of monosaccharide disintegration). As a result of acidic dehydration of monosaccharide and their subsequent condensation, there are formed pigmented oligomers consisting of 3-12 furan chains, containing numerous functional groups, such as carbonyl groups – (3.5-3.8) %, carboxyl groups – (2.8-7.1) %, phenolic hydroxyls – (1.0-6.0) %, aliphatic hydroxyl groups – (8.6-13.7) % and methoxy groups – (7.6-8.5) % and formed, as a result, insoluble joined polymers [1]. During hydrolysis there is also a peptization of part of low-polymeric lignin fractions to oligomers with a polymerization degree of 22-38, and a resultant molecular weight of 4000-7000 Dalton [2]. During the hydrolysate cooling process these lignin polymers distribute themselves between three phases – solid precipitate, colloidal and dissolved phases. Humic substances and intensively pigmented melanoids are referred to as difficultly oxidized high molecular weight impurities. These substances are produced and destroyed during hydrolysate preparation, fermentation, and at the subsequent biochemical industrial flow purification, but they still remain in waste waters.

Oxidation-resistant LHS removal from waste and recycling waters is an important problem since they are strong inhibitors of enzymatic and respiratory processes of yeast cells. Colloidal and dissolved LHS, contained in the substrate, do not readily penetrate into the endocellular space of yeast cells through the cytoplasmic membrane. Their inhibition for microorganisms is related to the interaction with cellular wall. During biochemical processing they are sorbed on the yeast cells surface, complicating their respiration and nutritional supply, which results in decreased enzymatic activity and negatively influences the processes connected with the biosynthesis of protein and nucleic acids, and to a lesser degree - polysaccharides and lipids. This is one reason for the low protein content in yeast which have been grown on poor quality substrates. Adsorption of negatively charged colloidal LHS is determined by the composition and structure of the yeast cellular wall, whose positive charge can be explained by the presence of chitin in it. The chitin, which supports the structure among other functions, actively sorbs anionic surface-active substances, and consequently LHS. Colloidal LHS, which don't penetrate through the chitin–glucan layer of the cellular wall, may be sorbed in the interporous space of the external cell layer. LHS sorption by yeast depends also on their concentration in the substrate which may reach 16 % of the yeast mass. Yeast which have been grown on industrial substrates containing LHS have  $\zeta$ -potential 20-30 mV, which is the reason for their decreased flotation ability [2]. LHS begin to influence enzymatic yeast activity at concentrations of 0.05-0.11 %, and increasing their concentration 0.20 % reduce the biomass yield of yeast *Candida scottii* and *Trichosporon cutaneum* to 28-40 % of the usually reduced substances. According to other data, increasing LHS concentration in substrate from 0.03 up to 0.30 % decreases the biomass yield by a factor of two [1].

In order to clarify polluted flows we used the treatment of flocculation. Industrial application of this method for treatment of hydrolysates or neutralizates of wood biomass for industrial flow has showed high effectiveness. As a flocculant we used a water soluble cationic polyelectrolyte (a co-polymer of dimethyldiallylammonia and vinyl-4-methylpyrrolidone). The optimum flocculant concentration is 25 mg/L. It was also found that flocculant addition influenced the  $\zeta$ -potential of waste water's LHS. So, for example, the optimum flocculant concentration results in changing of  $\zeta$ -potential of the dispersed phase from  $-32.6$  to  $+16.4$  mV, so that it actually gains positive charge [2]. LHS functional groups interact with amino groups of the flocculant in a stoichiometric manner much as weakly dissociated salts form.

To assay the LHS content quantitatively of both the dark (unclarified) and the clarified (treated) waste streams we used a method based on their adsorption by activated charcoal (SU-A), and subsequent ethanol and ammonium extractions. In parallel LHS determination was done spectrophotometrically at a wave length of 400 nm, using the method described in [2]. It was found that the LHS content in both flows are predominantly dissolved in ethanol fraction (60-76%). The main conclusion is that clarification by flocculation reduces the extractable LHS by 65.5%, and secondly that the more effective extractive agent is ethanol.

Table 1. Composition of oxidation resistant colloidal substances in waste waters

Lignohumic Substances (LHS)	Dark flows		Clarified flows		Effect of clarification, %
	LHS, mg/L	%	LHS, mg/L	%	
Soluble in $\text{H}_2\text{O}$	343	75.9	98	60.5	71.4
Soluble in $\text{NH}_4\text{OH}$	95	21.0	53	32.7	44.2
Insoluble residue	14	3.1	11	6.8	21.4
Adsorbed (sum)	452	100.0	162	100.0	64.2
Soluble LHS	438	96.9	151	93.2	65.5

For extraction and fractionation of the microheterogeneous impurities of waste waters (to investigate their dispersion profile) we used a microfiltration technique employing isotropic homoporous track membranes [3]. Due to their straight, cylindrical monodispersion pores, which are nearly perpendicular to the surface, this type of membrane is ideally suitable for LHS fractionation by the size.

In dark flows there are significant quantities of oxidation-resistant colloidal substances (average 356 mg/L). As for dispersed LHS, among colloidal impurities in dark and clarified wastes, the fraction with average particle diameter more than 667 nm is predominant. The flocculation treatment effectively removed substances with diameters more than 17 nm (average 78%). Furthermore the most finely-dispersed colloidal particles with diameter 17-100 nm are removed even better (84%).

## References

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