# Combined biological and physico-chemical treatment of baker's yeast wastewater including removal of coloured and recalcitrant to biodegradation pollutants

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**Abstract** The UASB reactor (35°C) was quite efficient for removal of bulk COD (62–67%) even for such high strength and recalcitrant wastewater as the cultivation medium from the first separation process of baker's yeasts (the average organic loading rates varied from 3.7 to 10.3 g COD/l/d). The aerobic-anoxic biofilter (20°C) can be used for removal of remaining BOD and ammonia from strong nitrogenous anaerobic effluents; however, it suffered from COD-deficiency to fulfil denitrification requirements. To balance the COD/N ratio, some bypass of raw wastewater should be added to the biofilter feed. The application of iron chloride coagulation for post-treatment of aerobic effluents may fulfil the discharge limits (even for colour mainly exerted by hardly biodegradable melanoidins) under iron concentrations around 200 mg/l.

**Keywords** Aerobic-anoxic biofilter; baker's yeast wastewater; iron coagulation; melanoidins; UASB reactor

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## Introduction

The baker's yeast industry is very popular in Russia. Such factories exist in almost all Russian provinces and altogether they produced 56 mln. $m^3$ wastewater per year (Kalyuzhnyi *et al.*, 2003). Since the main substrate for baker's yeast production in Russia is sugar beet molasses, these wastewaters are high strength (10–80 g COD/l), strong nitrogenous (0.5–1.5 g/l total N), sulphate-rich (2–10 g/l), phosphorus variable (sometimes P-deficient), recalcitrant for biodegradation and highly coloured (melanoidins etc.) ones. Currently many yeast factories are faced with heavy trade-effluent charges. Land disposal options generate problems with ground water pollution and are prohibited in majority of the Russian regions. Many local municipal sewage treatment plants are now insisting on pre-treatment of such effluents before discharge into their sewerage. The objective of this paper was to develop a lab scale technology for treatment of baker's yeast wastewater to meet the limits for discharge of treated wastewater into municipal sewerage. The most troublesome limits in this case are the following (mg/l, except colour): COD – 800; $SO_4^{2-}$ – 500; total N – 100; N-$NH_3$ – 50; P-$PO_4^{3-}$ – 3.5; colour threshold 1:128 (optical density < 0.2 at dominant wavelength). As a first treatment step, the UASB reactor operating at 35°C was applied for the elimination of the major part of COD and concomitant sulphate reduction. In a subsequent step, the biofilter operating in alternative aerobic-anoxic regime at ~20°C was used for the removal of the remaining BOD and nitrogen. Finally, iron coagulation was applied to fulfil the limits on COD, $PO_4^{3-}$ and colour.

## Materials and methods

### Wastewater

Since the major (~30% of total volume wastewater produced) and the strongest stream from

yeast factories (Kalyuzhnyi *et al.*, 2003) is the cultivation medium obtained after the first separation of yeasts (CM-1S), this stream taken from a Moscow baker's yeast factory is used in this study. The other reason is that this stream usually shows less variation in composition than the general effluent of yeast factories. Some characteristics of this wastewater during the period of sampling from November, 2002 to March, 2003 are given in Table 1. Gel-filtration of untreated WW (Figure 1a) revealed that the substances responsible for visible colour ($OD_{580}$) have symmetrical Gauss-type distribution of their molecular weights (MW) in the range of 0–30 kDa with a maximum at 6–7.8 kDa (>60% of colour is associated with the substances having MWs in the range of 2.6–14 kDa). On the contrary, substances with aromatic structures ($OD_{280}$) have non-symmetrical distribution of their MWs (Figure 1a) with a clear prevalence of low MW molecules (maximum at 2.6–4.5 kDa; > 82% of UV-absorption is associated with MWs < 8 kDa). These data show that the visible colour yeast wastewater is not closely associated with aromatics like phenolic compounds.

#### Gel-filtration

The gel Sephadex G-50 (Pharmacia, Sweden) was equilibrated with 0.1 N NaCl (pH 7.6) that was further applied as a mobile phase in a gel-filtration column. Potassium bichromate (fully permeating into gel) and blue dextran (fully non-permeating) were used for the calibration of the column. Based on elution volume ($V_e$) of calibration substances, the following equation for determination of MW ($V_e$ of bichromate and blue dextrane were assigned to column applicability limits – 1,500 and 30,000 Da, respectively) was obtained:

**Table 1** Range of variation of some characteristics of the CM-1S (average values from 5 samplings are given in brackets)

| $COD_{tot}$, g/l | $COD_{SS}$, g/l | $COD_{col}$, g/l | $COD_{sol}$, g/l | pH |
|---|---|---|---|---|
| 17.9–31.1 (22.5) | 0.95–2.93 (1.07) | 0.82–1.91 (1.46) | 15.0–26.6 (19.0) | 4.01–5.68 (5.14) |
| **Total N, mg/l** | **N-$NH_3$, mg/l** | **Total P, mg/l** | **P-$PO_4$, mg/l** | **$SO_4$, mg/l** |
| 993–1,651 (1,179) | 186–450 (278) | 12–78 (32) | 2–32 (9) | 682–3,028 (1,828) |
| **Phenolic compounds** | **Dominant wavelength, nm** | **Colour purity, %** | **Colour luminance, %** | **$OD_{580}$** |
| 713–1,167 (875) | 580 | 45.7–52.8 (48.6) | 40.6–50.2 (46.6) | 0.76–1.17 (0.95) |

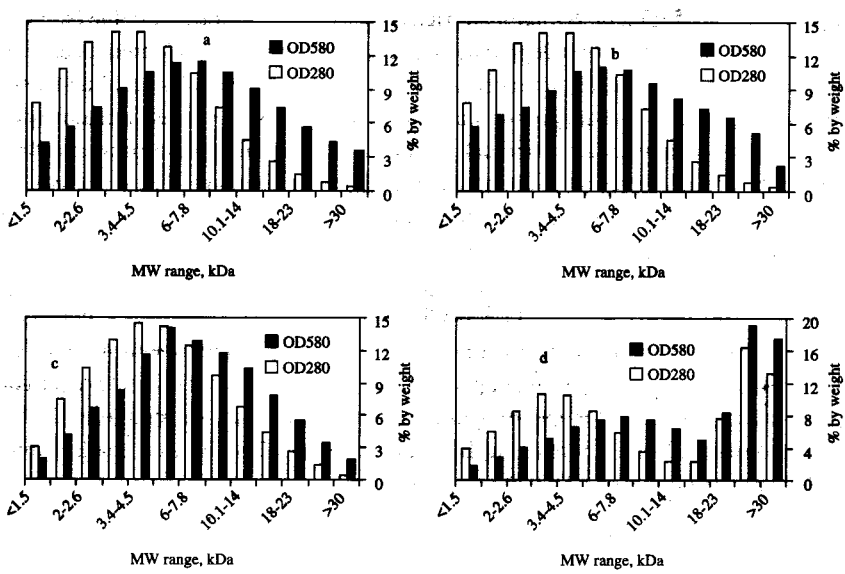


**Figure 1** MW distribution of substances exerting light absorption at 580 and 280 nm in raw CM-1S (a), anaerobic (b), aerobic (c) and final (d) effluents

$MW = \exp(12.521–0.1303V_e)$. During gel-filtration of untreated and treated wastewater, the eluting fractions (2.1 ml) were collected and their optical densities at 580 (dominant wavelength) and 280 (characteristic wavelength for aromatics) nm were determined.

#### UASB reactor

Laboratory UASB reactor (rectangular cross-section 33.64 $cm^2$, height – 82 cm, total working volume – 2.73 l) made from transparent plastic and equipped with 6 sampling ports along the reactor height was used. The reactor was kept in the thermostat (under 35 ± 1°C) and was seeded with granular sludge (66.5 g VSS, specific aceticlastic activity – 0.3 g COD/g VSS/day at 30°C) from the full-scale EGSB reactor treating brewery wastewater (Efes-Moscow). Assessment of sludge aceticlastic activities and determination of anaerobic biodegradability of the CM-1S were performed as described previously (Gladchenko and Kalyuzhnyi, 2003).

#### Anaerobic-anoxic bifilter

The tubular biofilter (diameter – 5 cm, height – 55 cm, packed by 0.5–2 cm fraction of road metal) had the working volume of 0.7 l and functioned in alternating aerobic/anoxic regime for treatment of the anaerobic effluents under ambient temperature in the laboratory (20 ± 3°C). The operation scheme included a sequencing process with a one-hour cycle consisting of 4 phases. During the first unfed phase, air at a flow rate of 0.8 l/min was pumped through an external loop of the biofilter. Aeration was switched off throughout the second unfed phase while the high recycle rate of effluent (0.125 l/min) was applied to ensure an adequate mixing and a complete consumption of the remaining soluble oxygen in the biofilter. During these 2 phases, nitrification and oxidation of remaining BOD proceeded. Then the feeding was performed during the 3rd phase under the same recycle rate of effluent. The last phase included only mixing (by effluent recycle) and was variable to close the 1 h working cycle of the programmable multi-channel timer controlling all 3 (air, recycle, feeding) pumps used. During the last 2 phases, denitrification proceeded. In the middle of the external loop of biofilter, an electronic sensor was inserted for on-line monitoring of soluble oxygen. The attached nitrifying-denitrifying biomass formed in the biofilter during the previous research (Gladchenko and Kalyuzhnyi, 2003) was directly used for treatment of anaerobic effluents in this study. The excess of sludge was periodically withdrawn by intensive backwash of biofilter.

#### Coagulation assays

They were performed with 200 ml of biofilter effluent in a laboratory glass under continuous stirring and pH control. Addition of coagulant ($FeCl_3 \cdot 6H_2O$) was carried out under 200 rpm, then intensity of stirring was reduced to 40 rpm to complete a flocculation process during which pH was maintained at 7.2–7.5 by addition of sodium hydroxide.

#### Analyses

All analyses were performed by *Standard Methods* (1995) or as described previously (Gladchenko and Kalyuzhnyi, 2003). All gas measurements were recalculated to standard conditions (1 atm, 0°C). Statistical analysis of data was performed using Microsoft Excel.

## Results and discussion

#### UASB reactor performance

In the preliminary experiments, it was found that the raw CM-1S's were quite biodegradable in anaerobic conditions (>90% on COD basis). Some results of the mesophilic UASB treatment (35°C) of the raw CM-1S's under quasi-steady state operation are shown in Table 2 (UASB runs). It can be seen that a stepwise decrease of hydraulic retention time

(HRT) from 4.9 to 2 days (organic loading rate (OLR) finally exceeded 10 g COD/l/d) almost did not influence total COD removal, which was in the range of 62–67%. Only traces of VFA were detected in the effluents (data not shown). However, such exhaustion of easily biodegradable COD in the anaerobic effluents might create COD deficiency problems for subsequent biological nitrogen removal. In spite of acidic influent pH feed, the effluent pH was close to 8 as a result of VFA consumption and mineralisation of nitrogenous species to ammonia (Table 2, UASB runs). The concentrations of phosphate increased (except run UASB-3) in the effluents (Table 2) due to mineralisation of phosphoric species. On the contrary, a gradual development of biological sulphate reduction led to an almost complete disappearance of sulphate in the UASB reactor (Table 2). The latter is almost quantitatively recovered as soluble sulphide in the sludge blanket zone of the reactor (data not shown). However, sulphide concentrations in the collected effluents were significantly lower due to its oxidation occurring in the settler zone of the UASB reactor and effluent collection vessel – the whitish precipitates (presumably, elemental sulphur) were clearly seen on the reactor and settler walls. Such losses occurred due to the small size of the laboratory reactor are hardly possible in full-scale industrial reactors. Colour removal was generally insignificant during this stage (Table 2).

Gel-filtration of anaerobic effluent (Figure 1b) also did not reveal significant changes in MW distribution of coloured and aromatic substances compared to influent (Figure 1a) indicating that these substances are persistent in anaerobic conditions. Throughout runs 1–3 (70 days), the quantity of sludge inside the UASB reactor almost doubled, its specific aceticlastic activity slightly increased and the VSS/TSS ratio slightly decreased (Table 3).

#### Performance of alternative aerobic-anoxic biofilter

In order to simulate a possible scenario for a yeast factory, when only the strongest stream (CM-1S) is treated anaerobically, then the effluent is mixed with the other less

**Table 2** Operational parameters and efficiency of the biological reactors (mean ± standard deviation)

| Parameter/run | UASB-1 | UASB-2 | UASB-3 | AeAnB-1 | AeAnB-2# |
|---|---|---|---|---|---|
| *HRT, days* | 4.86±0.08 | 3.28±0.03 | 1.98±0.11 | 3.11±0.07 | 4.35±0.44 |
| OLR, g $COD_{tot}$/l/d | 4.67±0.03 | 5.50±0.01 | 10.26±0.61 | 1.16±0.16 | 1.73±0.03 |
| Influent $COD_{tot}$, g/l | 22.33 | 17.93 | 20.25 | 3.59±0.48 | 7.9±0.04 |
| Effluent $COD_{tot}$, g/l | 7.57±1.07 | 6.63±0.62 | 6.72±0.12 | 1.15±0.17 | 2.30±0.02 |
| Total COD removal, % | 66.1±4.8 | 62.2±2.9 | 66.8±0.6 | 68.1±1.7 | 71.0±0.2 |
| Influent pH | 5.68 | 5.61 | 4.99 | 8.14±0.18 | 7.39±0.07 |
| Effluent pH | 8.16±0.11 | 8.17±0.09 | 7.95±0.10 | 7.83±0.1 | 7.63±0.12 |
| Influent $N_{tot}$, mg/l | 993 | 998 | 1075 | 474±10 | 636±11 |
| Effluent $N_{tot}$, mg/l | ND | ND | ND | 290±7 | 140±5 |
| Total N removal, % | – | – | – | 38.8±3.5 | 78.0±1.0 |
| Influent N-$NH_3$, mg/l | 202 | 186 | 235 | 416±21 | 558±3 |
| Effluent N-$NH_3$, mg/l | 702±25 | 783±12 | 729±30 | 25±5 | 64±5 |
| N-$NH_3$ removal, % | – | – | – | 94.0±0.9 | 88.5±1.0 |
| Effluent N-$NO_3$, mg/l | 0 | 0 | 0 | 207±31 | 12±2 |
| Effluent N-$NO_2$, mg/l | 0 | 0 | 0 | traces | traces |
| Denitrification efficien., % | – | – | – | 47.1±6.5* | 86.4±1.1* |
| Influent P-$PO_4$, mg/l | 2.3 | 1.8 | 5.6 | 3.2±0.8 | 7.7±0.1 |
| Effluent P-$PO_4$, mg/l | 11.1±1.6 | 4.8±1.5 | 4.2±0.1 | 3.1±1.2 | 4.1±0.8 |
| Influent $SO_4$, mg/l | 3028 | 1917 | 1245 | 17±15 | 164±3 |
| Effluent $SO_4$, mg/l | 448±76 | 41±25 | traces | 246±35 | 453±41 |
| Influent $OD_{580}$ | 1.11±0.09 | 0.76 | 0.755 | 0.38±0.01 | 0.53±0.01 |
| Effluent $OD_{580}$ | 0.86±0.14 | 0.735 | 0.73 | 0.33±0.01 | 0.41±0.01 |
| $OD_{580}$ removal, % | 22.4±6.4 | 3.3 | 3.3 | 11.2±0.9 | 22.5±1.2 |

*Calculated as: $\{1 - ([N\text{-}NO_3]_{ef} + [N\text{-}NO_2]_{ef})/([N\text{-}NH_3]_{in} - [N\text{-}NH_3]_{ef})\}*100$

#11% of raw CM-1S were added to balance COD/N ratio for denitrification

ND – not determined

**Table 3** Some sludge characteristics of a mesophilic UASB reactor treating the raw CM-1S.

| Parameter | Start | End |
|---|---|---|
| VSS in the reactor, g | 66.5 | 115.6 |
| TSS in the reactor, g | 101.6 | 181.2 |
| VSS/TSS, % | 65.5 | 63.8 |
| Aceticlastic activity, g COD/g VSS/day | 0.30 | 0.35 |



concentrated factory wastewaters and the UASB effluents were diluted (in ~2 times by tap water) before being fed to biofilter. After tuning of durations of the aerobic and anoxic phases, the following results were obtained (Table 2, run AeAnB-1). It is seen that the average total COD and ammonia removals accounted for 68% and 94%, respectively. However, the effluent nitrate concentrations were relatively high (207 mg N/l, on the average) that was related with COD deficiency to have a stable denitrification – some part of the incoming COD (~1.15 g/l, Table 2, run AeAnB-1) was non-biodegradable. To balance the COD/N ratio, some bypass of raw CM-1S (11%) was added to the biofilter feed during run AeAnB-2. This led to a substantial decrease of nitrate but some increase of ammonia in aerobic effluents (Table 2, run AeAnB-2). It seems that it is hardly possible to reach a lower level of ammonia in the effluent due to an immanent drawback of this relatively simple biofilter construction where wastewater filling and effluent withdrawal were performed simultaneously in a CSTR regime. Though during run AeAnB-2 the total inorganic nitrogen concentrations were around 76 mg N/l (Table 2), the aerobic effluents contained also significant concentrations (65 mg N/l) of organic nitrogen (seems to be hardly biodegradable) resulting in total nitrogen concentrations around 140 mg N/l, i.e., higher than the discharge limit to sewer (100 mg N/l). The total COD concentrations during run AeAnB-2 were close to the biodegradability limit of yeast WW but higher (Table 2) than discharge limit to sewer (800 mg/l). Due to oxidation of sulphide presented in anaerobic effluents, sulphate concentrations were below the discharge limit (500 mg/l); however, this was due to elementary sulphur pre-settling before biofilter treatment. Since such pre-settling may be difficult in implementation in full-scale conditions, sulphate can be a concern for biologically treated baker's yeast wastewater. The phosphate concentrations (Table 2, run AeAnB-2) in the aerobic-anoxic effluents were close to the discharge limit (3.5 mg P/l). In spite of 63% removal of phenolic compounds (data not shown) during the aerobic-anoxic stage, colour removal accounted for only 23% (Table 2, run AeAnB-2). This is in accordance with gel-filtration data for biofilter effluents (Figure 1c) when a shift of maximum of MW distribution of coloured substances to the range of 4.5–6 kDa and a decrease of relative content of low MW substances (< 2 kDa) was observed compared to untreated (Figure 1a) or anaerobically treated (Figure 1b) yeast wastewater. Thus, the visible colour is mainly associated with the other substances than phenolic compounds (e.g., persistent to biodegradation melanoids according to literature data (Francisca Kalavathi *et al.*, 2001)).

#### Performance of iron coagulation step

Some results on efficiency of the iron coagulation step for treatment of mixed aerobic-anoxic effluent are presented in Table 4. It is seen that all targeted parameters (total COD and nitrogen, phosphate, ammonia and colour) decreased with increasing acting Fe concentrations and the discharge limits are already achievable under iron concentrations around 200 mg/l. The colour of wastewater underwent dramatic changes from deep brown to pastel yellow after coagulation with 196 mg Fe/l. The gel filtration data for final effluent obtained (Figure 1d) also showed a substantial relative decrease of all fractions with MW < 18 kDa (colour-bearing fractions). A relative increase of fractions with MW > 18 kDa was due to

**Table 4** Performance of iron coagulation step

| | Acting Fe concentration, mg/l | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 99 | 196 | 476 | 909 | 1304 |
| $COD_{tot}$, mg/l | 1,310 | 1,110 | 800 | 690 | 550 | 400 |
| Phenols, mg/l | 234 | 208 | 107 | 96 | 50 | 44 |
| $P\text{-}PO_4$, mg/l | 4.7 | 3.1 | 1.4 | 0.1 | traces | traces |
| Total N | 120 | 101 | 82 | 63 | 34 | 25 |
| $N\text{-}NH_3$, mg/l | 34 | 25 | 16 | 10 | 5 | 3 |
| $OD_{580}$ | 0.325 | 0.298 | 0.170 | 0.071 | 0.021 | 0.014 |
| Sludge percentage, % vol*. | – | ND | 13.7 | 59.1 | 89.1 | 90.4 |
| SVI, ml/g TSS* | – | ND | 259 | 440 | 397 | 295 |
| Sludge VSS/TSS, %* | – | ND | 89.6 | 33.5 | 31.2 | 25.0 |

* after 30 min of settling
ND – not determined

formation of iron-induced aggregates with higher MWs having poor settling properties. These results are superior (with regard to coagulant added) to those reported in the literature for anaerobically treated baker's yeast wastewater (Kalyuzhnyi *et al.*, 2003). It is likely that the additional removal of COD (and partly colour) occurring on the aerobic-anoxic step led to a significant economy in coagulant addition. The sludge formed under an acting Fe concentration of 196 mg/l was relatively large (SVI = 259 ml/g TSS) and had high (~90%) VSS content (Table 2) showing the significant removal of organic COD and nitrogen during the iron coagulation step.

## Conclusions

1. The UASB reactor is quite efficient for removal of bulk COD (62–67% efficiency) even for such high strength wastewater as cultivation medium obtained after the first separation of yeasts.
2. The aerobic-anoxic biofilter can be used for removal of the remaining BOD and ammonia from anaerobic effluents; however, it suffered from COD-deficiency to fulfill denitrification requirements. To balance COD/N ratio, some bypass of raw wastewater should be added to the biofilter feed.
3. The application of iron chloride coagulation for post-treatment of aerobic-anoxic effluents may fulfill the discharge limits to the sewer under iron concentrations around 200 mg/l.

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