Environment Protection Engineering

Vol. 6 1980 No. 2

S. GWARDYS*, B. BACHMAN*

UTILIZATION OF BETAINE AND COLOUR SUBSTANCES IN YEAST FERMENTATION OF VINASSE AND BIOLOGICAL TREATMENT OF WASTEWATERS

The changes in concentrations of betaine and some groups of colour substances, contained in molasses wort subject to yeast fermentation with *Trichosporon cutaneum* and *Candida humicola*, were analysed. The loss of those substances in the separate stages of biological treatment of yeast factory wastewater was also determined. It has been found that betaine being not assimilated by yeast, however, is biodegradable, whereas the loss of colour substances both in the yeast fermentation and the biodegradation processes in only about 20% of their initial content.

1. INTRODUCTION

The wastewaters coming from production of spirit and yeast from molasses have similar chemical composition, although the concentrations differ. The estimated total volume of wastewaters produced in Poland annually amounts to ca. 6×10^6 m^3, and the pollutants load to 50×10^6 kg BOD_5 [8].

In the total mass of organic compounds betaine and colour sustances take an important position. They are introduced to technological processes of thb fermentation industry together with molasses as the basic substrate. The average concentration of betaine amounts to ca. 14%, and that colour substances — ca. 30% of the dry weight in the effluents from yeast factory and distillery [2].

One of the most important problem in the neutralization of distillery wastes was the utilization of molasses. At present it is fully utilized as the substrate for the production of fodder yeast. Unfortunately, however, the volume of the effluent after yeast fermentation of the vinasse is usually 2 times greater. Neutralization of this effluent is difficult and expensive as in the case of the vinasse not subject to yeast fermentation.

---

* Institute of Fermentation Technology and Microbiology, Technical University of Łódź.

In the available literature there are no data on the utilization of betaine and colour substances by yeast applied in the vinasse fermentation. The inadequate information about the transformations of these compounds during biological treatment of this kind of wastewater has inclined the authors to undertake the present work.

The purpose of the paper was to establish the degree of utilization of betaine and three groups of colour substances (products of alkaline degradation of invert sugar, caramel dyes and melanoidines) by *Trichosporon cutaneum* and *Candida humicola* and microorganisms participating in wastewater biodegradation.

2. CHARACTERISTICS OF BETAINE

Betaine may be presented as follows:

$$OH^{-}[(CH_3)_3 \equiv N-CH_2-COOH]^{+} \xrightarrow{-H_2O} (CH_3)_3 \equiv N^{+}-CH_2-COO^{-}.$$

In sugar beet there is about 1% of betaine. Its concentration depends on the variety degree of maturity, size of roots and sugar content.

Metabolic function of betaine in this plant is not exactly known. Most probably, owing to the presence of loosly bound methyl group, it may participate in transmethylation processes, i.e. in transfer of a methyl group from one compound to another [9].

In sugar production process almost total betaine passes to molasses, where its content amounts to about 5% of the dry weight whereas in vinasse and the effluents from yeast fermentation its concentration is as high as about 14% of dry weight.

3. CHARACTERISTICS OF COLOUR SUBSTANCES

Most colour substances present in molasses are formed during sugar beet processing. The amount and kind of those substances depend on individual features of the substrate and the method of the applied technological processes [6].

These substances are chiefly (in 80%) composed of the products of alkaline degradation of invert sugar. The colour of these substances is not intensive. Within this group glucynic, apoglucynic, molassic, and huminic acids deserve our attention [5].

Another group of colour substances (about 2.5% of the vinasse dry weight) is composed of the so-called caramel substances produced from monosaccharides and saccharose in dehydration process and in polymerization of dehydrated molecules at elevated temperatures (above 120°C). Their chemical structure is very little known.

The compounds classified among the group of melanoidines constitute about 2% of the vinasse dry weight. Melanoidines denote in general the colour substances formed in the Maillard's reaction from reducing sugars and amine compounds. This type of reaction takes place in all production stages, and its intensity can be efficiently inhibited by maintaining low concentration of sulphur dioxide [5].

The yeast and distillery wastes contain also amounts of the products of enzymatic oxidation of tyrosine and pyrocatechol, the so-called melanines. These are high-molecular compounds of the chinones nature.

The group of colour substances, i.e. complex compounds of iron and polyphenolic compounds, also deserves the attention.

Adding this to the substances formed from sugars and hydroxyacids, as well as dye stuffs formed in reactions of imidazole and indole compounds, one obtains a complicated system generally termed the colour substances.

The colour substances contained in vinasse subject to yeast fermentation have become of interest for technologists and microbiologists [10, 11]. The degree of interaction between microorganisms and the group of those substances contained in fermentation broth is studied.

4. THE INFLUENCE OF BETAINE AND COLOUR SUBSTANCES ON THE COURSE OF WASTEWATER TREATMENT

High concentrations of betaine in wastewater from yeast factory and distillery resulting from the biodegradation (especially in aerobic conditions) may lead to the formation of trimethylamine, according to the following reaction:

$$\underset{\text{betaine}}{\begin{array}{l} CH_2-COO^- \\ | \\ N^+(CH_3)_3 \end{array}} \xrightarrow[\text{activity}]{\text{bacterial}} \underset{\substack{\text{acetic acid}\\ \text{assimilated}\\ \text{by microorganisms}}}{CH_3-C\begin{array}{l} \diagup\!\!\diagup O \\ \diagdown O^- \end{array}} + \underset{\substack{\text{trimethylamine}\\ \text{not assimilated}\\ \text{by microorganisms}}}{N(CH_3)_3}.$$

The presence of trimethylamine in wastewater subject to furher treatment exerts an inhibitory effects on the metabolic activity of microorganisms.

HOCKENBURY and GREDY [4] studied the inhibitory effect of a number of organic compounds, including trimethylamine, on nitrification ability of activated sludge. They have stated that trimethylamine in the concentration of about 100 mg/dm^3 decreases this ability by 75%.

It is well known that when the hydrogen atoms at nitrogen are replaced by organic substitutents, then biochemical degradation of these compounds is difficult or impossible. Primary aliphatics are easily biodegraded, and aromatic amines biodegradation is more difficult, while that of tertiary amines does not practically exist.

The unpleasant decaying fish odour and its volatility (boiling point 2.9°C) adversely affects wastewater treatment practice.

The products of saccharose caramelization and melanoidines do not enhance the microbial growth either. High adsorption capacity of these compounds on the cell surfaces makes it difficult or even inhibits the growth of biomass. The amount of adsorbed compounds is directly proportional to their concentration in the solution and their maximum is reached at the isoelectric point which occurs within pH 2.5-3.0.

The colour of yeast wastes (dichromate-cobalt scale) is close to 10,000 mg Pt/dm^3, whereas the colour of vinasse (in 1:100 dilution) exceeds the intensity of the standard 250 mg Pt/dm^3. This intensive brown colour of wastewater is the prime difficulty in waste treatment, particularly when recycling is concerned.

5. METHODS

To establish the degree of betaine and colour substances utilization by yeasts in fermentation process, we have used molasses wort obtained by 1:1 dilution of vinasse with water supplemented with 0.35 g of urea and 0.35 g of diammonium phosphate in 1 dm^3. pH of molasse wort was corrected with sulphuric acid to about 4.5.

Yeast fermentation of molasses wort was conducted in 150 cm^3 shaken cultures at 30°C, 130 oscillations/minute, duration — 72 h (inocculation with pure culture — 2 g d.w./dm^3), and in fermentation tanks (fig. 1), culture volume — 3 dm^3, temperature —

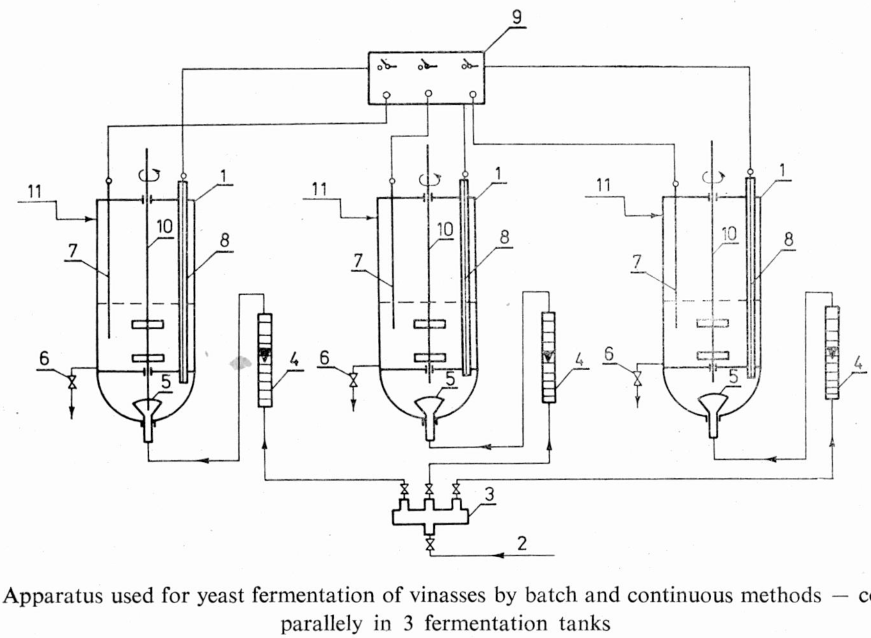

Fig. 1. Apparatus used for yeast fermentation of vinasses by batch and continuous methods — conducted parallely in 3 fermentation tanks

1 — fermentators, 2 — air supply, 3 — collector, 4 — rotameters, 5 — air distributors, 6 — drain valves, 7 — resistance thermometers, 8 — heaters, 9 — temperature controllers, 10 — stirrers, 11 — molasses wort supply

Rys. 1. Schemat aparatury stosowanej do zdrożdżowania brzeczki wywarowej metodą okresową i ciągłą równolegle w trzech fermentorach

1 — kadzie fermentacyjne, 2 — doprowadzenie powietrza, 3 — kolektor, 4 — rotametry, 5 — rozpylacze powietrza, 6 — zawory spustowe, 7 — termometry oporowe, 8 — grzałki, 9 — regulatory temperatury, 10 — mieszadła, 11 — doprowadzenie brzeczki

30°C, aeration 125 $dm^3/dm^3 \cdot h$, mixed at 600 rpm, batch duration — 3 days. In order to eliminate the influence of casual disturbances on the final effect, the culture was conducted parallelly in three identical fermentation chambers. In both types of cultures the pH of the medium was after every 24 h corrected with sulphuric acid, reducing to 4.5.

Samples of wastewater were taken from the sewer supplying the wastewater from yeast factory to the industrial wastes treatment plant and from the various points at the treatment plant, i.e.: from methane fermentation; after dilution and primary clarification; after biofilters; after oxidation ditches; effluent from biological ponds.

Samples were collected into glass flasks and kept before investigation at about 0°C for not longer than 4 hours.

Dry weight, ashes and pH were determined according to wastewater analyses manual [3]; betaine was determined colorimetrically [1]; and colour substances by the method of SAPRONOV [7], where the curves of spectral transmittivity in the near UV region are drawn. The concentrations of the individual groups of colour substances are determined from the extinction of the solution at the wavelengths corresponding to 250, 282 and 300 nm.

6. RESULTS AND DISCUSSION

Data in table 1 shows that the loss in dry weight of vinasse during its fermentation amounts to about 45% of its initial content.

Table 1

Utilization of betaine and colour substances in aerated cultures of *C. humicola* and *T. cutaneum* in vinesses (cultures conducted in fermentation tanks are shown in fig. 1)

Wykorzystanie betainy i ciał barwnych w hodowlach drożdży *C. humicola* i *T. cutaneum* w napowietrzanych brzeczkach wywarowych (hodowla prowadzona w fermentatorach pokazanych na rys. 1)

Specification	Units	Molasses wort before fermentation	Effluent after fermentation of molasses wort and separation of biomass	
			C. humicola	*T. cutaneum*
Total solids	g/100 cm^3	5.3	2.4	2.4
Ash	% TS	22.4	37.8	39.2
Organic substance	% TS	77.6	62.2	60.8
pH	—	4.5	7.6	7.7
Betaine	g/dm^3	5.0	4.8	4.8
Utilization of betaine	%	—	4.0	4.0
Products of invert saccharose decomposition	g/dm^3	11.9	9.3	9.4
Caramel dyes	g/dm^3	1.3	0.9	0.9
Melanoidines	g/dm^3	2.3	1.7	1.9
Total colour substances	g/dm^3	15.4	11.9	12.2
Percentage of colour substances utilization	%	—	22.7	20.8

Dry weight of the effluents from fermentation process consists of inorganic substances (35.5%) and organic compounds (61.5%). The loss of betaine in this process is as low as ca. 4%.

According to the authors this slight difference in the contents of betaine in vinasse subject to fermentation and in the effluent may be partially due to the errors of the method, but first of all to adsorption of this compound together with a certain amount of colour substances on the yeast cell surfaces.

Summing up, it should be stated that *T. cutaneum* and *C. humicola* are not able to assimilate betaine from the molasses wort. About 90% of betaine contained in molasses wort passes after fermentation to effluent, when it is a significant component of the organic substance.

The recorded changes in the concentration of colour substances in the cultures (both the shaken and the ones aerated in fermentation tanks) were similar. Therefore in fig. 2 and

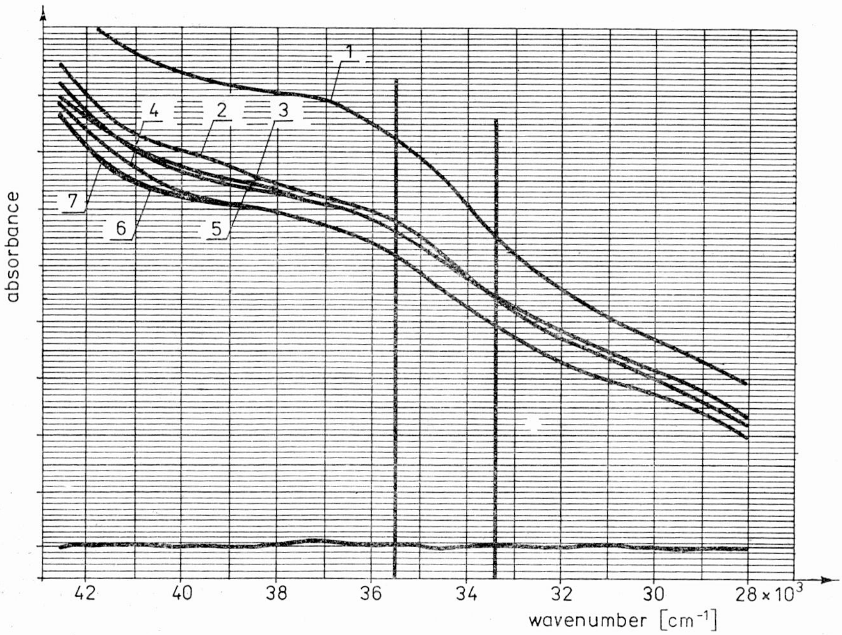

Fig. 2. UV adsorption curves

1 — for vinasse, 2-4 — for effluent after fermentation with *T. cutaneum*, 5-7 — for effluent from fermentation with *C. humicola* (dilution 100×)

Rys. 2. Krzywe adsorpcji w ultrafiolecie

1 — brzeczki wywarowej, 2-4 — odcieku po zdrożdżowaniu drożdżami *T. cutaneum*, 5-7 — odcieku po zdrożdżowaniu drożdżami *C. humicola* (rozcieńczenie 100×)

table 1 only these values have been specified which were obtained in cultures maintained in fermentation tanks with 20 times higher volumes than shaken flasks.

It should be noted that after 24 h of incubation, the curves of effluent adsorption before and after fermentation (fig. 2, curves 2 and 5) run much below the curve of the molasses wort adsorption (1), which corresponds to the decrease of the colour substances concentration by about 20% with respect to their initial content. In 2 days (curves 3 and 6) and 3 day cultures (curves 4 and 7) the loss in colour substances is, however, relatively small, amounting only to about 2%.

From the shape of the curves (fig. 2) it follows that the difference in the ability of both strains to utilize the colour substances is very small, being higher in *C. humicola* (curves 5-7).

Fig. 3 represents the scheme of yeast factory treatment plant operating in Poland. Results of analyses of wastewater samples taken at different treatment stages are given in table 2. During sampling the running parameters of this plant were kept within the limits corresponding to the normal operational standards.

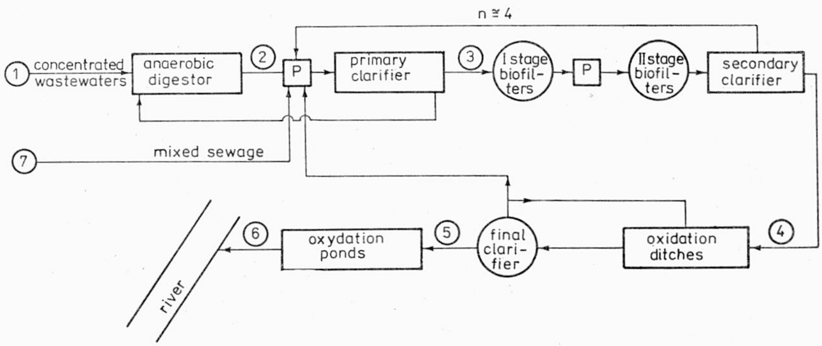

Fig. 3. Scheme of an industrial treatment plant for wastewater from yeast factory

1-7 — sampling sites

Rys. 3. Schemat przemysłowej oczyszczalni ścieków z drożdżowni

1-7 — miejsca pobierania próbek ścieków do badań

The changes in concentration of TS — total solids (dry matter residue), percentage of colour substances in TS and of betaine concentrations are presented graphically in fig. 4.

The wastewater discharged from the yeast plant is acidic (pH = 5.1), with 2.5% TS (table 2) of which about 67% fall to volatile organic solids (TVS). Betaine and colour substances take a large part of the TVS. The average content of betaine in concentrated wastewater is 3.8 g/dm^3 (table 2), of which 43% is biodegraded during anaerobic methane digestion, whereas the remaining 53% is utilized under aerobic conditions. After digestion the wastewater is diluted about 3.5 times with domestic sewage form the housing estate near the factory. Sewage contains only traces of betaine and colour substances. After dilution and further treatment on two-staged trickling filters betaine is completely removed from the wastewater.

Colour substances, in form of products of alkaline degradation of invert sugar (5.4 g/m^3), melanoidines (1.3 g/dm^3) and caramel dyes (1.1 g/dm^3), constitute about 30% of TS. This

Table 2

Results from analyses of samples after fermentation taken at different stages of biological treatment
Wyniki analiz próbek ścieków podrożdżowych pobieranych w różnych etapach oczyszczania biologicznego

Kind of sample	pH	Total solids	Ash		Betaine		Colour substances A*	Colour substances B*	Colour substances C*	Total A+B+C	
		g/dm^3	g/dm^3	% TS	g/dm^3	% TS	g/dm^3			g/cm^3	% TS
Wastewater from yeast factory	5.1	25.5	8.5	33.3	3.8	14.9	5.4	1.07	1.35	7.82	30.7
Wastewater after methane fermentation	6.2	13.2	6.3	47.7	1.6	12.1	3.0	0.64	1.21	4.85	36.7
Wastewater after dilution and primary settling tanks	7.2	2.6	1.2	46.1	0.3	11.5	0.5	0.07	0.20	0.77	29.6
Wastewater after biological filters	7.6	2.4	0.9	37.5	—	—	0.5	0.06	0.20	0.76	31.6
Wastewater after oxidation ditches	7.7	1.8	0.68	37.7	—	—	0.4	0.05	0.14	0.69	32.6
Effluent from oxidation ponds	7.9	2.0	0.60	30.0	—	—	0.4	0.04	0.12	0.56	28.0

A* — products of alkaline degradation of invert sugar, B* — carmel dyes, C* — melanoidines

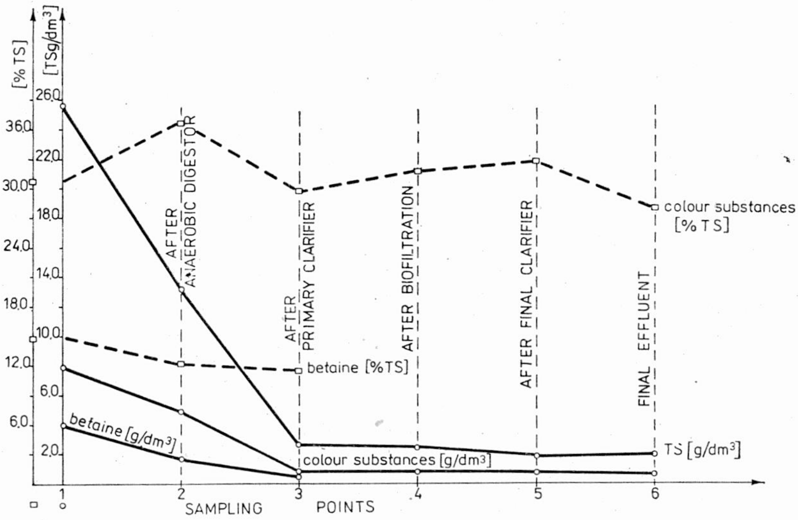

Fig. 4. Changes in concentrations of dry weight, betaine and colour substances in biological treatment of wastewater

1 — wastewater from yeast factory, 2 — after anaerobic (methane) digestion tanks, 3 — after dilution and primary clarification, 4 — after biofiltration filters, 5 — after oxidation ditches, 6 — wastewater after oxidation ponds (approx. 7 ha)

Rys. 4. Zmiany stężenia suchej masy, betainy i ciał barwnych w procesie biologicznego oczyszczania ścieków

1 — ścieki z drożdżowni, 2 — ścieki po wydzielonych komorach fermentacji metanowej, 3 — ścieki po rozcieńczeniu i wydzieleniu osadów w osadnikach wstępnych, 4 — ścieki po złożach biologicznych spłukiwanych, 5 — ścieki po rowach utleniających, 6 — ścieki po stawach biologicznych o powierzchni około 7 ha

significant group of organic compounds is utilized to small extent by microorganisms both in aerobic and anaerobic conditions. The largest removal from 7.82 g/dm^3 to 4.85 g/dm^3 takes place in anaerobic digestion. Further decrease from 4.85 to 0.77 g/dm^3 is achieved through dilution with domestic sewage and after the clarification. Subsequent treatment hardly lowers the concentration of these substances any further.

The effluent from oxidation ponds has an intensive brown colour and contains about 0.6 g/dm^3 of colour substances.

From the curves shown in fig. 4 it follows that the loss of TS during biodegradation is quicker than that of colour substances, especially in the first stages of treatment.

Wu Yen and Kao Chiao [12] present their own results and discuss the results obtained by Londong, stating that the utilization of colour substances in microbiological processes is not efficient. The loss in concentrations of those substances, according to them, does not exceed several percent.

Summing up, the utilization or removal of colour substances from industrial distilleries and yeast plants effluents is very important to which not enough attention has been paid so far. The same is true for physico-chemical methods for colour removal from such wastewaters, especially adsorption on activated carbon which shows excellent affinity for colour causing substances [13].

7. CONCLUSIONS

1. Glycine betaine contained in molasses is not asimilated by baker's yeast — *Saccharomyces cerevisiae*; *T. cutaneum* and *C. humicola* (cultured as a fodder biomass) do not show either any ability to assimilate betaine from molasses wort.

2. During wastewater treatment about 42% of betaine is removed in the anaerobic digestion (mesophilic methane fermentation), and the remaining 52% during aerobic biofiltration. Wastewater after treatment in biological filters does not contain betaine.

3. In *T. cutaneum* or *C. humicola* cultures conducted in vinasses the utilization of colour substances ranges from 20 to 22% of their initial concentration. The remaining part (ca. 80%) is discharged with wastewater.

4. In the yeast factory treatment plant about 20% of colour substances is also removed in the anaerobic digestion tanks. The remaining colour substances (together with wastewater) pass throught remaining treatment stages being neither biodegraded nor adsorbed.

5. During biological treatment of wastewater from yeast factory (fig. 3) decolourization of wastewater is not achieved. Thus it seems advisabe to look for other methods for the removal of this group of compounds exceptionally resistant to biodegradation.

REFERENCES

[1] CROMVEL B.T., RENNIE S.O., Biochem. Journal, No. 1 (1953), p. 189.
[2] GWARDYS S., Zeszyty Naukowe Politechniki Łódzkiej, Rozprawy Naukowe, No. 22 (1979).
[3] HERMANOWICZ W., DOŻAŃSKA W., DOJLIDO J., KOZIOROWSKI B., *Fizyczno-chemiczne badanie wody i ścieków*, Arkady, Warszawa 1976, p. 714.
[4] HOCKENBURY R.M., GRADY L.P., Journal Water Pollution Control Federation, No. 5 (1977), p. 768.
[5] MCGINNIS R.A., *Cukrownictwo*, WNZ, Warszawa 1976, p. 70.
[6] PREY V., ANDERS H., BRAUNSTEINER W., HOLLE H., Z. Zuckerind, No. 7 (1971), p. 323.
[7] SAPRONOW A.R., Saharnaja prom., No. 8 (1963), p. 82.
[8] SKALSKI K., TOMCZYŃSKA J., N.O.T., Rok nauki polskiej, Materiały pokonferencyjne, Warszawa 1973.
[9] STEINMETZER W., Zucker, No. 2 (1972), p. 48.
[10] ŠWEC W.N., KNOGOTKOWA E.I., ŠAŠENKOW E.F., Izwestija Wysšyh Učenyh Zawedenij, No. 5 (1977), p. 49.
[11] TAEWSKAJA L.W., USENKO W.A., Ferm. spirt. prom., No. 7 (1976), p. 79.
[12] WU YEN C., KAO CHIAO F., Journal Water Pollution Control Federation, Vol. 48 (1976), p. 2609.
[13] ZAORSKA H., Gazeta Cukrownicza, No. 9 (1966), p. 209.

WYKORZYSTANIE BETAINY I CIAŁ BARWNYCH W PROCESACH ZDROŻDŻOWANIA WYWARU I BIOLOGICZNEGO OCZYSZCZANIA ŚCIEKÓW

Zbadano zmiany stężenia betainy oraz niektórych grup ciał barwnych zawartych w brzeczkach wywarowych poddanych zdrożdżowaniu drożdżami *Trichosporon cutaneum* i *Candida humicola*. Określano również ubytek tych substancji w poszczególnych etapach biologicznego oczyszczania ścieków z drożdżowni.

Stwierdzono, że betaina nie jest asymilowana przez drożdże, natomiast ulega biodegradacji w procesie oczyszczania ścieków, zaś ubytek ciał barwnych, zarówno w procesie zdrożdżowania, jak i w toku biologicznego oczyszczania ścieków, wynosi tylko około 20% ich początkowej zawartości.

DIE VERWERTUNG VON BETAIN UND FARBSTOFFEN BEI DER VERHEFUNG VON SCHLEMPEN UND BEI DER BIOLOGISCHEN ABWASSERREINIGUNG

Untersucht wurden Konzentrationsänderungen von Betain und mancher Farbkörpern aus Absudschlempen, die einer Verhefung mittels der Hefeart *Trichosporon cutaneum* und *Candida humicola* unterzogen wurden. Bestimmt wurde auch der Abbau dieser Substanzen in den einzelnen Stufen der biologischen Reinigung der Abwässer aus Hefefabriken.

Man konnte feststellen, daß Betain von Hefen nicht assimiliert wird, aber biologisch abbaubar ist; die Abnahme von Farbkörpern sowohl bei der Verhefung sowie bei der biologischen Abwasserreinigung beträgt i.M. nur 20% des Eingangswertes.

ИСПОЛЬЗОВАНИЕ БЕТАИНА И ЦВЕТОВЫХ ТЕЛ В ПРОЦЕССАХ ДРОЖЖЕВАНИЯ БАРДЫ И БИОЛОГИЧЕСКОЙ ОЧИСТКИ СТОЧНЫХ ВОД

Были исследованы изменения бетаина, а также некоторых групп цветовых тел, содержащихся в бардяных суслах, подвергнутых дрожжеванию дрожжами *Trichosporon cutaneum i Candida humicola*. Определена убыль тех веществ в отдельных этапах биологической очистки сточных вод от дрожжевого завода.

Было выявлено, что бетаин не ассимилируется дрожжами, а подвергается биодеградации в процессе очистки сточных вод, убыль же цветовых тел как в процессе дрожжевания, так и в ходе биологической очистки сточных вод составляет около 20% их начального содержания.