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Reduction of the Contents of Carbon, Phosphorus and Nitrogen in Waste Products from the Alcohol Industry by the Method of Denitrification

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Abstract

This paper reports results of a study on the reduction of the COD in waste products from the alcohol industry based on the denitrification process, with the use of the laboratory culture of *Bacillus licheniformis* bacteria. The optimum conditions for the process have been established, taking into account the effect of the composition and dilution of the waste, temperature of the process, concentration of nitrates and phosphates, and aeration. In optimum conditions COD was reduced by over 70%, and phosphorus by about 60%. The total reduction of nitrates was obtained in 24 hours.

Keywords: reduction of chemical oxygen demand (COD), waste products from the alcohol industry, denitrification, *Bacillus licheniformis* bacteria.

Introduction

The production of alcohol at Polish distilleries converted to 100" spirit was 133 million litres in 1997 [1]. The raw products for alcohol production are potatoes, rye, molasses or pirosulphite liquor. The composition of the wastes coming from the process is determined by the raw products used. For alcohol production on the industrial scale molasses - a byproduct of sugar production - is most often used. It contains about 50% of soluble and non-crystallising sugars and is a cheap raw product for obtaining alcohol. Processing 1 ton of molasses gives about 2.5 tons of waste, which means that in a year we get 700,000 tons of wastes [2] characterized by high contents of organic compounds (COD varies from 55,000 to 75,000 mg O_2/dm^3) and potassium (5000-10,000 ppm). A significant concentration of colloidal compounds is responsible for its turbidity, and the presence of humus compounds for its brown colour. The composition of the wastes makes it impossible to discharge it to municipal waste purification plants and excludes the possibility of passing it to open waters.

The typical composition of the waste product is given in Table 1 [3].

The waste product of molasses is most often processed by yeast cultivation using mainly the yeast Candidia and Trichosporan and getting about 20 g of dry yeast mass from 1 litre of the waste liquid. The yeast assimilate mainly sugars, glycerine, free aminoacids and volatile organic acids, leading to the reduction of COD to a few thousand mg O_2/dm^3 [4]. However, also after this reduction it is impossible to mix these wastes with municipal ones. Unfortunately, many alcohol-producing plants pass the waste products to the municipal sewage system and pay fines, sometimes exceeding the profit. In view of the above, the neutralization of these wastes seems to be of utmost importance.

One of the methods for dealing with waste involves its concentration and combustion, however, this method is not welcome by local inhabitants because of the dust and very unpleasant smell.

A promising method for reduction of the content of organic compounds and phosphorus in waste is biological

Table 1. The typical composition of waste after molasses processing.

pH	~ 5
dry mass [g/dm^3]	5.7 – 9.2
parameter	% of dry mass weight
organic compounds	71 – 83
organic acids (lactic, glycolic, malic, tartaric, glucomic, oxalacetic)	4.7 – 27
sugars (fructose, glucose, sacharose)	2.5 – 2.7
aminoacids (glutamic, aspartic, alanine, seryne, glicyne)	1.7 – 10.6
glicerine	5.2 – 15.5
betaine	6.7 – 15.3
N total	2.5 – 6.0
P_2O_5	0.1 – 2.3
parameter	ppm
B vitamins	100
K	5300 – 10000
Na	130 – 1200
Ca	700 – 1700
Si	240 – 300
Fe	48 – 62
Mg	42 – 68
Zn	28 – 36
Cu	8 – 16
SO_4^{2-}	~ 50

denitrification. The bacteria use for their metabolism a wide spectrum of organic compounds which become for them the source of energy and carbon for synthesis of cell structures. The compounds they metabolise include: sugars, alcohols, organic acids and aminoacids. These compounds undergo oxidation and the released electrons are transported along the subsequent elements of the respiration chain to nitrates, which are the final electron acceptors. Phosphorus is mainly used in synthesis of high energy intracellular compounds (ATP, ADP). Thus, as a result of denitrification the concentrations of the main three eutrophising agents: C, P and N are considerably reduced. This paper reports results of the study on optimisation of the process of removal of organic carbon and phosphorus, from the molasses and rye-molasses processing waste from the plant in Kolaczkowo, near Gniezno, using the method of biodenitrification.

Materials and Methods

Microorganisms

Bacillus licheniformis bacteria were grown on a modified Starkey medium [5] at 37˚C in tightly closed reactors of 20 ml in volume into which 10 ml of inoculated medium was introduced. The inoculum, making 4% vol. of the medium, was collected on the average after 20 h.

Analytical Methods

Nitrates: potentiometric method, ion-selective electrode with an AgCl reference electrode — made by Detector [6].

Nitrites: spectrophotometric method with sulphanilamide and N-(l-naphtylo)-ethylenodiamine hydrochloride [7], Beckman DU 640.

Orthophosphates: spectrophotometric method, absorbance by phosphomolybdate blue [8], Beckman DU 640.

COD: determination of bichromate oxidation, Close Reflux Titrametric Method [9], cells 16/100 mm, made by Merc.

Protein: the Lawry method [10], Beckman DU 640.

C, N, H: Elemental analyser Perkin Elmer 2400.

Metals: K, Ca, Mg, Fe, Na: the ASA method, ELA spectrophotometer, Unicam SP90A.

Sulphates: method based on precipitation of $BaSO_4$ [11].

Preliminary preparation of the wastes:

The two kinds of wastes obtained after molasses and rye-molasses (20% rye and 80% molasses) processing, denoted by A and B, respectively, were provided by the plant in Kolaczkowo, near Gniezno. Before they could be subjected to denitrification, they required the following procedures:

- correction of pH to 7.2-7.8,
- centrifugation, a centrifuge MPW-330, 3500 rotations/min, time of centrifugation 5 minutes,
- dilution, the waste A (molasses) had to be diluted at ratio of 1:1, waste B (rye + molasses) was used without dilution,
- introduction of phosphates in the amount ensuring a con centration not lower than 100 mg $P\text{-}PO^{3-}_4/dm^3$,
- introduction of nitrates to the value optimum for the process,
- aeration of the whole volume of the waste sample, mem brane pump "Skalar 1-2".

Table 2. Characteristics of raw wastes

Parameter [unit]	Raw waste A (molasses)	Raw waste B (molasses-rye)
pH	4.2	3.5
density [g/cm^3]	1.03	1.04
dry mass [g/dm^3]	21	21
protein [g/dm^3]	10.1	8.0
COD [mgO_2/dm^3]	70,000	46,000
$P\text{-}PO_4^{3-}$ [g/dm^3]	0.035	0.430
$N\text{-}NO_2^-$ [g/dm^3]	0.000	0.000
$N\text{-}NO_3^-$ [g/dm^3]	0.0	0.0
$S\text{-}SO_4^{2-}$ [g/dm^3]	0.145	0.046
Fe [ppm]	66	15
Mg [ppm]	0.90	0.180
K [ppm]	25,000	25,000
Ca [ppm]	925	950
Na [ppm]	900	750
C [% d.m.]	35	39.4
N [% d.m.]	4.3	3.9
H [% d.m.]	6.2	6.0

Results represent the average values obtained from three parallel series, consisting of three samples each.

Results and Discussion

Identification of the composition of raw wastes Chemical analysis of the raw wastes was made and their basic properties were determined, the results are given in Table 2.

Because of particularly high COD values, waste A had to be diluted. In its non-diluted form denitrification was very slow and incomplete (non-reduced nitrates and nitrites were left). In subsequent experiments the waste was diluted at ratios of 1:1, 1:2 and 1:4. Already when diluted at the ratio of 1:2, denitrification was complete and run at a satisfactory rate. Greater dilutions did not improve the kinetics of the process. The concentrations of inorganic salts met the requirement of the denitrificators so there was no need to supplement them [12, 13]; the only exception was a too low concentration of phosphates in waste A (molasses). According to the results of our further experiments, the best reduction of COD is achieved when the concentration of phosphates is not lower than 100 mg $P\text{-}PO_4/dm^3$.

The Effect of Temperature

The effect of temperature on the degree of COD reduction is illustrated by the data in Table 3.

At 22°C, even after 90 hours of incubation, about 300 mg of $N\text{-}N0_2^-$ was left in the medium. When temperature was raised to 28°C and 37°C, already after 24 hours denitrification was complete. Final parameters of the waste were very similar for both temperatures. Further increase of temperature caused a prolongation of the process, and at 44°C no growth of denitrificators was observed.

The Effect of Nitrate Concentration

The degree of COD reduction as the result of denitrification significantly depends on the concentration of the nitrates introduced, as illustrated by the data from Table 4 and Figs. 1 and 2.

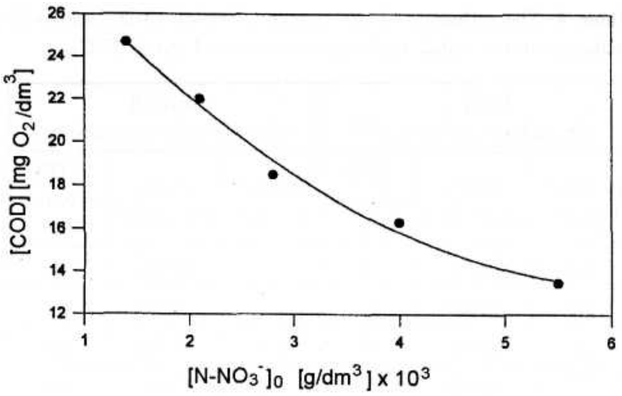

Fig. 1. The effect of the concentration of nitrates on COD reduction in waste A (after molasses processing, dilution 1:1, 37°C).

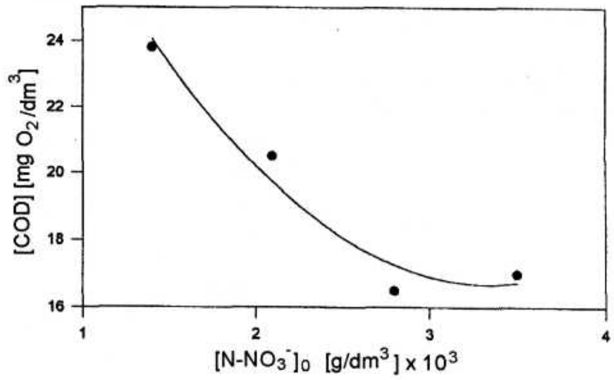

Fig. 2. The effect of the concentrations of nitrates on COD reduction in waste B (after rye-molasses processing, undiluted, 37°C).

The degree of COD reduction in waste A (after molasses processing) increases to over 60% with the concentration of nitrates increasing to 5.5 g $N\text{-}NO_3^-/dm^3$. However, at this concentration the process is prolonged from 24 to 48 hours. When nitrate concentration is further increased, even after 170 hours of the process, there is a considerable amount of unreduced nitrates and nitrites left in the medium. In waste B (rye-molasses) the degree of COD increases to 64% with the concentration of nitrates increasing up to 2.8 g $N\text{-}NO_3^-/dm^3$, when the latter concentration is

Table 3. The effect of temperature on the degree of COD reduction in the two kinds of tested wastes (2.8 g $N\text{-}NO_3^-/dm^3$).

	Waste A (after molasses processing) 1:1				Waste B (after processing of rye-molasses mixture)			
	22°C	28°C	37°C	44°C	22°C	28°C	37°C	44°C
COD before denitrification [mgO_2/dm^3]	incomplete denitrfication	35,000	35,000	incomplete denitrification	incomplete denitrification	46,000	46,000	incomplete denitrification
COD after denitrification [mgO_2/dm^3]		20,000	21,100			16,500	16,000	
degree of COD reduction [%]		43	40			64	64	
time [h]		24	24			36	36	

Table 4. The influence of the concentration of nitrates on COD reduction in the tested wastes (process carried out at 37°C).

Waste A (after molasses processing 1:1)			Waste B (after rye-molasses processing)		
$[N\text{-}NO_3^-]_0$ [g/dm³]	$[COD]_r$ [mg O_2/dm³]	COD reduction [%]	$[N\text{-}NO_3^-]_0$ [g/dm³]	$[COD]_r$ [mg O_2/dm³]	COD reduction [%]
1.4	24,700	30	1.4	23,800	48
2.1	22,000	37	2.1	20,500	55.5
2.8	18,500	47	2.8	16,500	64
4.0	16,300	53.5	3.5	17,000	63
5.5	13,500	61.5	4.0	incomplete denitrification	
6.5	incomplete denitrification				

Table 5. The effect of phosphates concentration on COD reduction in waste A (37°C).

$[N\text{-}NO_3^-]_0$ [g/dm³]	COD [mg O_2/dm³]		
	0.050 g PO_4^{3-}/dm³	0.100 g PO_4^{3-}/dm³	0.260 g PO_4^{3-}/dm³
1.4	incomplete denitrification	23,400	26,200
2.1		21,700	22,500
2.8		18,000	18,500
4.0		16,100	16,300
5.5		13,600	13,500
6.5		15,000	14,500

Table 6. Reduction of COD under the effect of aeration of the molasses processing waste after denitrification (dilution 1:1, 2.8 g $N\text{-}NO_3^-$/dm³, 37°C).

$[N\text{-}NO_3^-]_0$ [g/dm³]	COD [mg O_2/dm³]			COD reduction [%]
	before aeration	after 2.5 hours of aeration	after 5 hours of aeration	
2.8	18,500	13,100	13,000	29
4.0	16,700	10,500	10,000	37
5.5	13,500	8,500	8,500	37

Table 7. Reduction of COD in the recycled waste left after molasses processing (dilution 1:1, 37°C).

$[N\text{-}NO_3^-]$ [g/dm³]	Process duration [h]	$[COD]_r$ [mg O_2/dm³]	COD reduction [%]
2.8 + 2.8	72	10,800	69
2.8 + 2.8 + 2.8*	incomplete denitrification		
2.8 + 2.8 + 1.4*	120	9,600	73
4.0 + 4.0	incomplete denitrification		

* the variant containing 2.8 g $N\text{-}NO_3^-$/dm³ was after recycling inoculated for the third time with addition of 2.8 or 1.4 g $N\text{-}NO_3^-$/dm³

reached duration of the process increases from 24 to 36 hours. Further increase in nitrate concentration leads to incomplete denitrification.

The Effect of Phosphate Concentration

Frequently, the concentration of phosphates is the factor limiting denitrification [12]. In a subsequent series of experiments we checked for different concentrations of nitrate to determine what concentration of phosphates is required for complete denitrification. Since the concentration of phosphates in waste B (rye-molasses) is 0.430 g $P\text{-}PO_4^{3-}$/dm³ and exceeds that in the standard medium (0.215 g $P\text{-}PO_4^{3-}$/dm³), the effect of phosphate concentration was studied only for waste A (molasses), which in the raw state contained only 35mg $P\text{-}PO_4^{3-}$/dm³. The results are given in Table 5.

As follows, the presence of phosphates at the concentration of 0.1 g $P\text{-}PO_4^{3-}$/dm³ is not a factor limiting the process of denitrification, even at very high concentrations of nitrates (5.5 g $N\text{-}NO_3^-$/dm³). A greater than two-times increase in phosphate concentration does not improve the kinetics of the process, while a decrease in their concentration leads to the incomplete denitrification, even at the lowest concentration of nitrates (1.4 g $N\text{-}NO_3^-$/dm³).

Aeration of the Wastes

In order to improve the effect of denitrification on the reduction of COD , the wastes after denitrification were subjected to aeration. The relevant results are given in Table 6.

According to the data from Table 6, aeration of waste A (molasses) for 2.5 hours, after completion of denitrification, leads to further reduction of COD from 14 to 18% of the value obtained by denitrification. The 2.5 h aeration of waste A in which 5.5 g $N\text{-}NO_3^-$/dm³ underwent denitrification, resulted in the total reduction of COD of 77%. The increase of the aeration time to 5 hours did not lead to a further reduction of COD.

Table 8. The optimum conditions for the reduction of COD in the tested wastes in the process of denitrification.

Parametr	Waste A (after molasses processing) 1:1	Waste B (after processing a mixture of rye-molasses)
temp. °C	28 – 37	28 – 37
pH	7.2 – 7.8	7.2 – 7.8
$[N\text{-}NO_3^-]_0$ [g/dm³]	4.0	2.8
$[N\text{-}NO_3^-]_r$ [g/dm³]	0	0
$[N\text{-}NO_2^-]_r$ [g/dm³]	0	0
retention time [h]	24	36
aeration time [h]	2.5	–
COD_{final} [mg O_2/dm³]	10,500	16,500
reduction COD [%]	70	64
$[P\text{-}PO_4^{3-}]_0$ [g/dm³]	0.100	0.430
$[P\text{-}PO_4^{3-}]_r$ [g/dm³]	0.034	0.210

Aeration of waste B (rye-molasses) after denitrification did not give any further reduction of COD, probably this kind of waste contains compounds which are more difficult to oxidize than those in waste A.

Recycling of the Wastes

After denitrification (2.8 and 4.0 g $N\text{-}NO_3^-/dm^3$) wastes were again inoculated, their pH adjusted and the contents of phosphates and nitrates supplemented. The variant with 2.8 g $N\text{-}NO_3^-/dm^3$, after recycling was inoculated for the third time, adding 2.8 or 1.4 g $N\text{-}NO_3^-/dm^3$. In samples of the waste B (rye-molasses) for each of the tested nitrate concentrations, after recycling both nitrates and nitrites were left unreduced. Probably, after recycling, the waste samples are deficient in the components necessary for the functioning of denitrificators. The results of this series of experiments are presented in Table 7.

The maximum reduction of COD is obtained for waste A (after molasses processing) containing 2.8 g $N\text{-}NO_3^-/dm^3$, recycled with added 2.8 g $N\text{-}NO_2/dm^3$ and recycled again with added 1.4 g $N\text{-}NO_3^-/dm^3$. In this case the final COD was 9600 mg O_2/dm^3, which corresponds to its 73% reduction. However, the time of this full process in this case is extended to 120 hours.

On the basis of the results obtained, taking into regard the time of the process and degree of COD reduction , we proposed the optimum conditions for the successful denitrification of the wastes from the alcohol production industry (Table 8).

An important finding is that the process of denitrification also leads to a decrease in the content of phosphorus, one of the main elements causing eutrophication of water reservoirs, by 54% and 66% in wastes B and A, respectively. The final 70% reduction in COD to the value of about 8000 mg O_2/dm^3 allows the wastes to be passed to municipal sewage system and further purified in municipal plants. The proposed method of using biodenitrification for purification of the wastes highly loaded with organic compounds eventually leads to obtaining technological water or wastes purified to meet accepted standards. The method is very promising, and we expect to achieve further reduction of COD by combining the processes of denitrification and desulfurication under appropriate aeration conditions.

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