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PIGGERY WASTEWATER FERMENTATION BY YEAST**

Results of direct yeast fermentation of raw wastewaters from a large piggery are presented. Only four *Candida* type yeasts proved robust enough to withstand the conditions of an unbalanced substrate such as pig wastes. Various methods of primary hydrolysis were investigated and discarded as uneconomical. Enrichment of raw wastes with molasses has been found not feasible because of increased residual COD contamination of centrate after incubation, and high costs. Yeast cells yields were above 2 kg d.w./m^3 for all species grown on raw wastes; the *Candida tropicalis* strain 8 showed the highest yields.

1. INTRODUCTION AND SCOPE OF WORK

Large scale animal farms, with an industrialised production process, with adjoining fodder preparation and mixing plants, seem to ofter an opportunity for recycle and recovery due to the sheer economy of scale. Methods of utilization of animal wastes can be divided into several categories:

1. Utilization of sludge resulting from conventional treatment of wastewaters for stream discharge;
2. Direct agricultural utilization of wastewaters and / or sludge;
3. Direct extraction of valuable components by concentration, separation;
4. Conversion of unwanted components into a more valuable form.

Since the piggery wastewaters from large farms are very dilute [1] the methods of direct extraction and conversion to methane and Single Cell Protein (SCP) have not as yet been applied in full scale.

Yeast fermentation for SCP has been extensively used by industry utilizing such waste products as whey, molasses and spent sulphite liquors. Wastewaters from numerous food industry effluents have been successfully fermented by *Candida utilis*, *Saccharomyces*

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cervisiae, *Trichoderma viride*, *Rhodotorula glutinis*, and other species into single cell protein in laboratory and pilot scale studies [2]. Concentrated piggery wastes have been fermented only in laboratory, sometimes with the addition of cracked corn [3] or with addition of other carbohydrate substrates such as sucrose [4] in fungi fermentation by *Aspergillus niger*.

The ultimate goal of the presented research is evaluation of economic feasibility of producing pig feed supplement consisting of yeast derived from the farm wastewaters — through fermentation. The system, outlined recently [5], could ultimately close the nutrient cycle in the farm, and produce high quality protein and low concentration liquid effluent for stream disposal — after adequate polishing treatment.

This paper presents the initial results of the screening tests designed to evaluate:

— feasibility of fermentation of dilute manure;
— eventual need for carbohydrate supplementation;
— selection of the group of the most promising organisms;
— delineation of further pilot scale research priorities.

2. METHODS AND EQUIPMENT

Initially, screening tests of a large number of yeast species obtained from the Institute of Fermentation Industry in Warsaw were performed. The tests consisted of growing yeasts on agar — solidified piggery wastes in microbiological vials. The four species selected from these tests, i.e. *Candida tropicalis*, strain 11 and strain 8, *Candida robusta* and *Candida utilis* strain 3 have proved their growth potential on the manure substrate (other species failed to reproduce) and are characterized by intensive growth rate and high protein content of good quality — when grown in optimum conditions. It should be noted that other strains, such as *Torula casei*, *Torulopsis candida* or *Rhodotorula glutinis* have not survived the pig manure substrate; at the same time the four selected species proved only 30% survival, as determined by Löffler blue staining vitality test.

Further screening of the initial preparation methods and species was performed in batch tests. These consisted of various methods of hydrolytic breakdown of cellulolytic materials to bring up the energetic potential of the piggery wastewater prior to fermentation. Since various authors recommend different variations of preparatory treatment [7] the following methods of hydrolysis were tested in the batch mode:

1. Sulphuric acid hydrolysis (pH 1.0) at 1.5 atmosphere in an autoclave for 2 hours;
2. Sulphuric acid hydrolysis at atmospheric pressure;
3. Heating on boiling water bath for 2 hrs;
4. Decanting the manure, evaporation, acid hydrolysis and dilution to previous concentration.

The preparation of manure through hydrolysis was aimed at increasing the carbon to nitrogen C/N ratio in respect to easily biodegradable carbohydrate carbon. In the batch tests that followed, hydrolysis was not used. The reason is evident when one

compares the total sugar content after preparatory treatment, table 1 with raw untreated manure. The raw wastewater concentration of total sugars varied between 200–530 mg/dm^3 while hydrolysis by the various methods, outlined above, yielded total sugar content of 230 to 740 mg/dm^3. The addition of molasses of up to 2% boosted total sugars to over 19,000 mg/dm^3.

Because of these results, all subsequent work was done on untreated raw piggery wastewaters. As a routine, the wastewaters were brought to pH 5–5.5 with sodium hydroxide, put into 250 cm^3 beakers, sterilized, seeded with some 50 cm^3 of monoculture seed grown on brewery mash and incubated for 3–5 days in 303 K.

The beakers were aerated with warm air, through sterile cotton air filters, in thermostatic incubators. If foaming became excessive, a few drops of sterile soya oil were added (fig. 1).

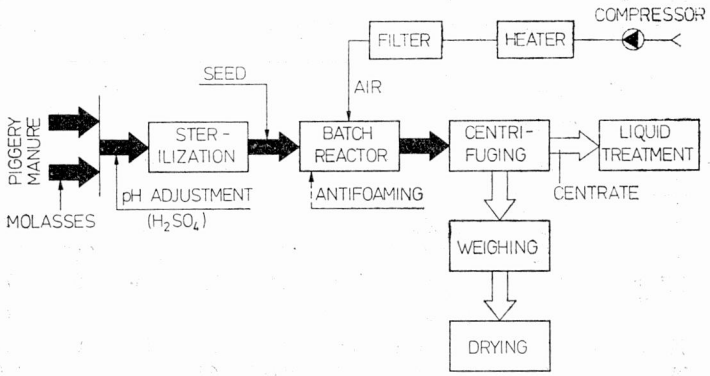

Fig. 1. Layout of batch experiment procedures

Rys. 1. Schemat układu laboratoryjnego

After incubation the samples were centrifuged at 3000 rpm, coke was weighed, dried, ground, weighed again and analyzed. The weight of the centrifuged manure sludge from a sterile reference sample and of the seed was substracted from the total obtained in order to get the true net microbial mass yield; supernatant was also analyzed.

All analyses were performed according to the US EPA guidelines and Standard Methods [6] except for the colorimetric anthrone determination of the energetic material present — performed in accordance with Polish Standards PN-76/C-04628/02 and except for the vitality Löffler blue staining test.

3. RESULTS

The following results are confined to the analysis of two substrates: raw untreated pig manure — with pH adjusted to 5.5 and raw untreated manure enriched with molasses with pH adjusted to 5.5. No hydrolysis was used for pretreatment because the process was considered not feasible in full scale.

The tests were run on two parallel batches of wastewaters (with and without molasses) each in four units to accommodate all four *Candida* type yeasts. The results of these eight combinations are presented in table 1, which provides raw wastes centrate data, centrate (effluent) quality data and percent removals of individual parameters. Thus, table 1 serves as an estimation of the potential of yeast fermentation in treatment of soluble fraction of piggery wastes. This is better illustrated in fig. 2 where influent and effluent COD and Total Kjeldahl Nitrogen concentrations are compared.

Table 1

Results of batch fermentation of raw centrate of piggery effluent by yeast of *Candida* type
Efekty zastosowania drożdży typu *Candida* do fermentacji surowych ścieków z tuczarni świń

Parameter	Unit mg/dm³ as	Untreated wastes	Molasses enriched	*C. robusta* 1		*C. tropicalis* 11		*C. tropicalis* 8		*C. utilis* 3	
		A	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*
COD	O_2	4136	19666	1382	5821	1270	7738	1382	8656	1428	13318
TKN	N	650	1348	261	496	261	373	250	550	276	739
N–NH_3	NH_4^+	288	500	219	76	193	45	183	78	186	103
N–NO_2	NO_2	t	t(trace)	t	t	t	t	t	t	t	
N–NO_3	NO_3	1.1	16.4	t	t	t	t	t	30	t	35
N–org.	N_2	421	848	68	420	68	328	67	472	91	736
$PO_4^{\equiv}$ =	$PO_4^{\equiv}$	453	464	303	17	232	30	284	25	236	175
Tot. P	P	315	338	187	25	265	17	86	21	273	93
Sugars	$C_6H_{12}O_6$	500	19600	417	58	420	1760	460	48	468	1336
Removals (%) of:											
COD				67	70	69	61	67	56	65	32
TKN				60	64	60	72	62	59	57	45
N–NH_3				24	85	33	90	36	73	84	36
N–Org				84	50	84	61	83	44	78	13
Phosphates				34	96	49	93	37	94	48	62
Total P				40	92	16	96	73	94	13	73

Table 1 shows raw and yeast treated centrate data because the only way to remove yeasts, commercially, would be by centrifuge. The tests were run on untreated total wastewaters. The cake from the centrifuge after fermentation consists of manure solids, seed solids and biomass culture; the actual values, compiled in table 2 were calculated by subtracting from the total cake mass the masses of seed and the manure solids. The table also lists units cells yield calculated from the difference between the influent centrate concentrations of TKN (nitrogen according to Kjeldahl method) and carbon (calculated as 25% of COD) and their respective effluent concentrations.

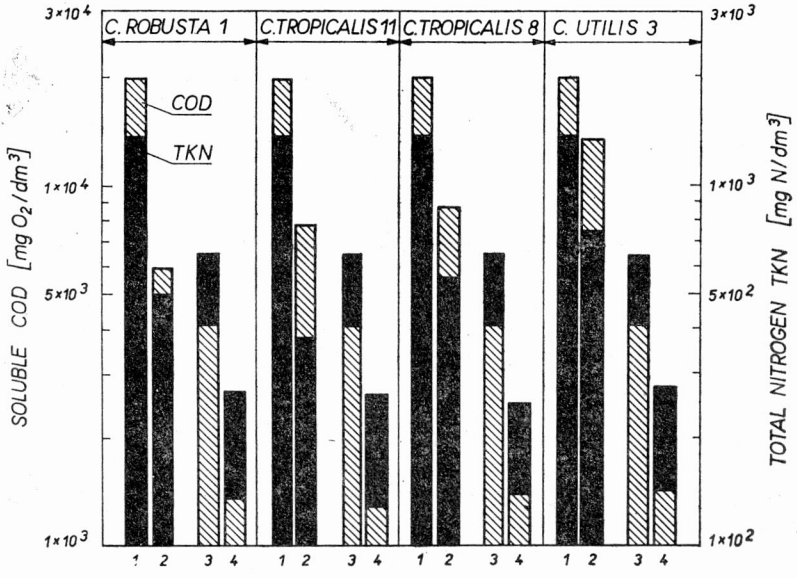

Fig. 2. COD and TKW removals in batch fermenters
1 — influent, *2* — effluent; raw untreated wastes, *3* — influent, *4* — effluent

Rys. 2. Usuwanie ChZT i TKN w dozującym zbiorniku fermentacyjnym
1 — przed oczyszczaniem, *2* — wyciek; nieoczyszczone surowe ścieki, *3* — przed oczyszczaniem, *4* — wyciek

Table 2

Yeast	Biomass yield in yeast fermentation							
	Yeast yield kg d.w./m³		Unit cells yield kg cells/kg nutrient removed					
			Nitrogen		Carbon		Phosphorus	
	Raw	Enrich.	Raw	Enrich.	Raw	Enrich.	Raw	Enrich.
C. robusta 1	2.13	5.86	5.5	4.6	3.1	1.7	16.6	18.7
C. tropicalis 11	2.22	7.58	5.7	5.8	3.1	2.5	44.4	80
C. tropicalis 8	2.74	6.11	6.8	4.8	4.0	2.2	12	19
C. utilis 3	2.05	2.85	5.5	2.3	3.0	1.8	49	8.4

4. DISCUSSION

Results in table 1 indicate good COD removals by all species of the *Candida* yeasts. The **raw wastes** COD removals oscillate closely in the range of 65–69%. The nitrogen removals expressed in TKN show a range of 57–62%, similarly N_{org} removal is stable. Phosphorus removals seem to favour *C. tropicalis* 8 (73%) and *C. robusta* 1 (40%) against *C. tropicalis* 11 (16%) and *C. utilis* 3 (13%), although the latter well removes $N-NH_3$

(84%). The data on fermentation of untreated raw wastes for soluble pollutant removal indicates the suitability of the first two species mentioned.

The data for **enriched wastes treatment** clearly discriminates between the four species in favour of *C. robusta* 1 with the highest COD removal (70%) and constantly high removals of other nutrients, 64–96%. Definitely second best seems to be *C. tropicalis* 11, followed by *C. tropicalis* 8, while *C. utilis* yields the poorest removals, 13–73%.

Analysing the batch fermentation from the biomass production angle, in table 2, it becomes apparent that the two *C. tropicalis* give the best cell yields in both raw (2.22 and 2.74 kg dry weight/m^3) and in enriched wastes. The interpretation of unit cells yield reveals much better utilization of nitrogen, carbon and phosphorus in case of raw wastes. This means that enrichment with molasses does not improve the unit yields, i.e. kg cells/kg N, P, C removed, and that it leaves a considerable energy potential in the soluble centrate — in all cases exceeding the raw (untreated) centrate COD value.

An important point should be stressed here, namely the initial ratios of C/N and C/P = 3.3 and for enriched C/N = 3.64, C/P = 14.5, are much below the average optimum ratios practiced in the fermentation industry. The reason lies in the unusually high nitrogen and phosphorus content of piggery wastewaters, and leads to the contention that without carbon enrichment yeast fermentation will always retain a lot of nutrients in the soluble effluent.

The analysis of data in table 2 indicates a somewhat better performance of *Candida tropicalis* strains 8 and 11 over *C. robusta* 1 and *C. utilis* 3, both on enriched and raw wastes. The unit cells yield more and favour the two *C. tropicalis* strains, however the margin is not large.

Preliminary estimates of protein content based on TKN · 6.25 data reveals dry weight protein content from approx. 46% (*Candida utilis* 3) to over 60% (*C. tropicalis* 11).

5. CONCLUSIONS

1. The preliminary hydrolysis to increase the carbohydrate content of piggery wastes proved ineffective and will not be feasible in full scale.

2. The solidified substrate tests have screened out four *Candida* type strains: *C. robusta* 1, *C. tropicalis* 11 and 8, *C. utilis* 3.

3. The batch liquid fermentation for 120 hours at an optimum pH 5.5 yields good removals of COD from the soluble fraction of wastewaters, i.e. 65–69% for all species and TKN removals of 57–62%, for raw wastes without enrichment.

4. The enrichment of piggery wastewaters results in:

— production of biomass apparently more lively than raw unadjusted wastes;

— production of larger biomass yield (kg/m^3), however, at lower efficiency (kg cells/kg nutrient);

— increased removal of nitrogen and phosphorus from soluble effluent.

5. The results show that enrichment with molasses is not technically and economically feasible, because:

— enrichment does not increase the unit cells yield;
— retains considerable concentration of COD and certain amount of nutrients;
— technical cost of supplying waste molasses are forbidding;
— raw unsupplied wastes yield good cells production.

6. Considering the influent and effluent C/P, C/N ratios and their respective changes it is concluded that raw, soluble piggery wastewaters are an unbalanced medium for aerobic aseptic fermentation.

7. The biomass of all yeast species, after drying and grinding reveals no trace of specific pig manure odour.

8. Out of the four screened species the *Candida tropicalis* 8 was the most suited for piggery wastes treatment and biomass production, while *Candida utilis* 3 showed the poorest performance.

9. Preliminary contents estimation in the dry biomass reveals high protein content ranging from 46 to 61%, d.w.

Further work is presently conducted on optimization of operational parameters in dynamic yeast fermentation, in pilot scale. Current batch fermentation research is aimed at the development of fungal biomass on pig wastes.

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DROŻDŻOWANIE ŚCIEKÓW Z TUCZARNI

Przedstawiono wstępne wyniki drożdżowania ścieków z wielkich tuczarni przemysłowych. Z przebadanych szczepów drożdży jedynie cztery: *Candida utilis* 3, *Candida tropicalis* 8 i 11, *Candida robusta* 1 wytrzymały warunki trudnego substratu, jakim jest niezrównoważona pod względem składu surowca gnojowica z tuczu trzody chlewnej. Przebadano różne metody hydrolizy wstępnej substratu i stwierdzono jej nieprzydatność z przyczyn technicznych, technologicznych i ekonomicznych. Wzbogacanie w węglowodany melasą odpadową okazało się nieprzydatne ze względu na silny wzrost stężenia resztkowego ChZT w centracie po 120 godzinnej fermentacji oraz ze względu na wysokie koszty. Średnie uzyski drożdży wynosiły 2 kg s.m./m^3 ścieków, przy czym *Candida tropicalis* 8 wykazała najwyższy przyrost masy (2.74) oraz najlepszy przyrost jednostkowy z azotu — 6,8 kg s.m./kg N usuniętego.

ZUR VERHEFUNG VON ABWASSER

Besprochen werden die Erstversuche der Verhefung von Abwässer aus Tiermastbetrieben. Von mehreren eingesetzten Hefestämmen, nur vier: *Candida utilis* 3, *Candida tropicalis* 8 und 11 sowie *Candida robusta* blieben in der schwankenden der Zusammensetzung nach und schwer abbaubaren, rohen Schweinegülle beständig. Untersucht wurden verschiedene Methoden der Hydrolyse des Substrats, wobei die Unbrauchbarkeit dieses Verfahrens aus technischen, technologischen und wirtschaftlichen Gründen festgestellt wurde. Eine Anreicherung mit Kohlehydraten in Form einer Melassegabe erwies sich sowohl wegen hohen Kosten als auch wegen hohen CSB-Werten im Zentrat, nach 120 Stunden Faulung, unbrauchbar. Die durchschnittliche Ergiebigkeit der Verhefung betrung 2 kg TS Hefe/m³ Abwasser, wobei *Candida tropicalis* 8 mit 2,74 kg TS/m³ bzw. 6,8 kg TS/kg N-Abbau an der Spitze lag.

ДРОЖЖЕВАНИЕ СТОЧНЫХ ВОД

Представлены предварительные результаты дрожжевания сточных вод, образующихся в крупных откормочных хозяйствах. Из обследованных штаммов дрожжей только *Candida utilis* 3, *Candida tropicalis* 8 и 11, *Candida robusta* перенесли условия трудного субстрата, каким является несбалансированная по составу жижа от откорма свиней. Проверены разные методы предварительного гидролиза субстрата и обнаружена непригодность его по техническим, технологическим и экономическим причинам. Обогащение углеводами помощью мелассы оказалось непригодным из-за сильного роста остаточной концентрации химического содержания кислорода (хск) в центрате после 120 часов брожения, а также из-за высокой стоимости. Средний выход по дрожжам составлял 2 кг с.м./м³ сточной воды, причем *Candida tropicalis* обнаружила наиболее высокое приращение массы (2,74), а также наилучшее удельное приращение от азота — 6,8 кг с.м./кг удаленного N.