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EFFECTS OF A CARBON SOURCE ON EFFECTIVENESS OF YEAST PROPAGATION IN STICKWATERS *)

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Key words: *Candida* yeast, stickwaters, propagation of yeast, fish oil.

Propagation of five selected strains of *Candida* yeast in stickwaters after fish meal production and stickwaters with added carbohydrates and crude fish oil was investigated. The highest level of propagation was reached in stickwaters supplemented with 2% of fish oil.

INTRODUCTION

The production of fishmeal with the two-step wet method renders large guantities of stickwaters which are dumped as sewage after degreasing and partial separation of solids. The main components of stickwaters include proteins and non-protein nitrogen compounds. The non-protein nitrogen accounts here for 43% [13]. Chemical coagulation of the proteins with inorganic salts may help to recover up to 40% of total nitrogen compounds [14]. The deproteinized stickwaters still retain an average of ca 4,5% nitrogen compounds, and most of the vitamins, mineral salts, and microelements. Separation of these components, physically or chemically, is complicated and unprofitable. The organic nitrogen compounds and other substances present in the stick waters considerably hamper the operation of waste-treatment plants and constitute a serious threat to ecology.

One of the most effective ways of utilizing waste, stickwaters, and industrial sewage water is to use them as substrates in production of single cell proteins [3, 7, 9, 10, 12]. In effect, we can obtain biomass good as fodder, and reduction of levels of substances having negative

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properties in sewage. Taking into consideration the composition and volume of the stick waters resulting from production of fish meal, it was deemed useful to try and recover the substances by propagation of yeast. The latter could increase the fish meal yield as well as its biological value. Sewage from fish meal plants could be partially purified. It was also understood that the investigations could reveal the possibilities of using stickwaters as a source of nitrogen and growth substances in the case of yeast propagation in substrates containing only a source of carbon.

MATERIALS AND METHODS

MICROORGANISMS

Strains of *Candida lipolytica* and *Candida tropicalis* were obtained from Institute of Fermentation Industry in Warsaw; *Candida lipolytica* CX 161-1B(A) — from Institute of Biochemistry and Biophysics (Warsaw), and *Candida* sp. 4 and 5 were isolated from fish. The strains were selected from thirty samples in the collection and in fish and other material from a fish meal plant. They were active lipolytically and proteolytically and a clear case of correlation between their enzymatic activity and growth intensity in stickwaters was observed.

MEDIUM

It consisted of defattened and deproteinized stickwaters [14], diluted to 4 mg/cm^3 nitrogen total contents and supplemented with 0,2% admixture of KH_2PO_4. The growth-stimulating carbon source was an addition of glucose and molasses (3%) or crude fish oil (2%). The levels of nitrogen, carbohydrates oil, and phosphorus were determined as optimal for growth of the investigated strains.

CULTURES

The medium (pH 4,5 to 4,7) was inoculated with 3-day old yeast cultures from a peptone and glucose medium. The inoculum was introduced at 5% level in ratio of the medium volume (0,05 to 0,06 g dry weight) 100 cm^3. The cultivation was continued up to the maximum propagation of the population, at 30°C in a shaker (120 rpm) in 300 cm^3 flasks containing 50 cm^3 of the medium. The growth was checked by measuring the optical density at $\lambda = 660$ nm, after prior extraction of the oil with a mixture of ethanol, butanol, and chloroform, in the case of cultures in the presence of fish oil [4].

ANALYSES

The total contents of nitrogen and fractions precipitated with 4% and 15% TCA were determined with Kjeldahl method; aminoacid nitrogen — after a modified formolic method after Sörensen; carbohydrates — after Dubois [2]; lipids — Soxhlet extraction method after acidic hydrolysis, according to Weibull-Stoldt; chlorides — after Mohr. The level of protein was calculated from the total quantity of nitrogen determined with the Kjeldahl method multiplied by 6,25. Dry weight of the biomass and the medium as well as the contents of mineral compounds were determined routinely. The biomass output was calculated in ratio of total dry weight of the medium minus mineral compounds unused by the yeast.

RESULTS AND DISCUSSION

PROPERTIES OF STICKWATERS AS A MEDIUM FOR YEAST PROPAGATION

Table 1 presents the characteristics of deproteinized stick waters. Following precipitation of a part of protein, the stickwaters retain ca 4,5% nitrogen compounds, of which ca 56% is non protein nitrogen and only 15% is aminoacid nitrogen, most easily assimilated by yeast. Thus, the main component of stickwaters are peptides and proteins, soluble in 4% TCA and coagulated by 15% TCA. The quantities of carbohydrates and lipids as potential sources of carbon are rather low in defated stickwaters. This is due to low levels of carbohydrates in fish meat and their rapid

Table 1. Composition of deproteinaized stickwaters after fish meal production

		Deproteinized stickwaters	Dilute stickwaters	$S = \frac{\sigma}{\bar{x}} \cdot 100\%$ for deproteinized stickwaters
Total nitrogen	mg/cm³	7,20	4,00	20
Protein nitrogen (precipitated using 4% TCA)	mg/cm³	0,85	0,48	105
Non-protein nitrogen (soluble in 15% TCA)	mg/cm³	4,10	2,26	58
Amino acids nitrogen	mg/cm³	1,08	0,61	23
Dry weight	g/100 cm³	7,07	3,96	40
Minerals	g/100 cm³	2,95	1,65	98
Lipids	g/100 cm³	0,30	0,17	56
Carbohydrates	g/100 cm³	0,10	0,06	10
Chlorides	g/100 cm³	1,85	1,04	98

Mean-values determined with 25 different stickwaters.

T a b l e 2. Results of yeasts propagation using stickwaters without added carbon source and stickwaters with crude fish oil addition

	Yield of biomass g dry weight/dm³		Yield of biomass % of dry matter		Utilization of total nitrogen %		Utilization of dry matter %		Protein in biomass %		Protein g/dm³/h	
	1	2	1	2	1	2	1	2	1	2	1	2
Candida lipolytica	8,5	23,4	37	54	21	38	35	51	54	43	0,15	0,34
Candida lipolytica A	8,9	21,5	38	50	18	30	33	47	53	40	0,16	0,27
Candida tropicalis	7,9	24,0	34	56	20	40	35	51	55	43	0,15	0,35
Candida sp. 4	8,3	20,9	36	48	18	29	21	47	52	40	0,15	0,27
Candida sp. 5	8,3	23,6	36	55	20	38	34	50	53	42	0,15	0,34

1 — stickwaters
2 — stickwaters + fish oil
The yield of dry biomase expressed in percent of dry matter of the medium without minerals

The coefficient of variation $S = \frac{\sigma}{\bar{x}} \cdot 100\%$ for the yield of biomass fluctuated from 10 to 24% [1, 2].

transformation under the influence of tissue and bacterial enzymes. The stickwaters are characterized by considerable variation of the contents of particular forms of nitrogen and mineral compounds, mainly salts. The variability of composition of stickwaters stems from the variaties of the raw material available, its kind, freshness, preservation methods, and the fish meal production parameters.

PROPAGATION OF YEAST IN STICKWATERS

Some strains of yeast assimilate nitrogen compounds as a source of carbon, and for this reason, the first attempts at propagating yeast were carried out in stickwaters only. The average values obtained from 8 different stickwaters are given in Table 2. The maximum propagation of populations of the investigated strains took place after 24 to 36 hours, depending on the types of stickwaters. After conclusion of the cultivation process 7,9 to 8,8 g of dry weight cm^3 was obtained with 20% utilization of nitrogen and 30% to 35% utilization of the dry weight of the medium. Considering the quantities of nitrogen absorbed from the medium and from the volume of the biomass, it can be said that the used strains utilized organic compounds of nitrogen as a source of carbon, yet from the practical point of view the effectiveness of the process was low. Further experiments were, therefore, conducted with stickwaters supplemented with glucose (a standard), molasses, and fish oil, as possible technological sources of carbon.

EFFECTS OF ADDED CARBOHYDRATES ON GROWTH OF YEAST

Table 3 presents the results of propagation of yeast in 10 different samples of stickwaters supplemented with carbohydrates. Addition of glucose or molasses led to double or triple increases of the biomass volume, better utilization of nitrogen, and almost complete consumption of sugar. The guantity of biomass stickwaters with added molasses was 10% to 20% lower than in the case of the use of glucose. It was correlated with a lower quantity of nitrogen absorbed from the medium and with a greater variability of the process. The most productive strains rendered 20 to 23 g of dry weight yeast from 1 dm^3 of the medium (25% to 50% utilization of nitrogen compounds; 32% to 36% on the average). These results correspond with reports by others who propagated yeast in wastes with carbohydrates as a source of carbon. The stickwaters remaining after separation of proteins from vegetable juices rendered 14 to 20 g dry weight yeast from 1 dm^3 following cultivation for 24 to 60 hours, depending on the strains and medium used in the process [9, 12]. Using of whey in most cases also renders 20 to 23 g of dry weight of product [7]. We can say, therefore, that stickwaters with carbohydrates

Table 3. Results of yeasts propagation using stickwaters with carbohydrates addition

	Yield of biomassa g dry weight/dm^3		Yield of biomass % of dry matter		Utilization of total nitrogen %		Utilization of carbohydrates %		Protein in biomass %		Protein g/dm^3/h	
	1	2	1	2	1	2	1	2	1	2	1	2
Candida lipolytica	21,8	18,4	41	35	36	31	95	95	53	50	0,15	0,11
Candida lipolytica A	22,5	18,7	42	35	33	29	96	95	51	51	0,14	0,11
Candida tropicalis	18,8	14,7	35	33	30	30	28	97	50	51	0,12	0,10
Candida sp. 4	22,8	20,7	43	39	30	30	95	95	46	48	0,13	0,12
Candida sp. 5	23,0	20,6	43	39	34	32	96	95	51	50	0,15	0,13

1 — stickwaters + glucose
2 — stickwaters + molasses
The yield of dry biomass expressed in percent of dry matter medium without minerals.

The coefficient of variation $S = \frac{\sigma}{\bar{x}} \cdot 100\%$ for the yield of biomass fluctuated from 20 to 24% [1] and from 27 to 32% [2].

provided a relatively high yield of biomass after some 70 hours of cultivation.

EFFECTS OF FISH OIL GROWTH OF YEAST IN STICKWATERS

The results of growth in 10 samples of stickwaters supplemented with 2% crude fish oil are given in Table 2. All strains assimilated lipids as a source of carbon and energy more easily in comparison with carbohydrates and reached the maximum multiplication within 24 to 36 hours, depending on the composition of the stickwaters (most often it ocurred after 30 hrs). One dm^3 of medium rendered 18 to 29 g of biomass dry weigth, 23 to 24 g on the average, depending on the type of stickwaters. With ca 50% utilization of the medium dry weight and complete processing of the added oil, on the average about 40% of nitrogen compounds were recovered as protein. This equaled ca 30% lowering of the contents of dry weight in stickwaters. Fish oil, one of the components of stickwaters, turned out to be a much more profitable supplementary source of carbon than carbohydrates.

The volumes of biomase received from stickwaters with fish oil are equal or higher than those reported by other authors. Van der Veen, who used *Endomycopsis lipolytica* and a mineral medium with 2% waste fats, obtained between 10 to 13 g biomass dry weight from a single dm^3 within 28 to 30 hours (10). Langridge and Templer collected 12 g biomass dry weight after 48 hrs of cultivation on a medium with 1% fish oil (8). Hottinger et al: (5, 6) reported that their strains *Candida lipolytica* and *Geotrichum candidum* rendered the highest levels of biomass when the medium contained 5% fish oil. After 48 hrs they obtained under optimal conditions 28 g biomass *Candida lipolytica* and 31 g biomass *Geotrichum candidum* from one dm^3 medium. Absolute quantity of biomass collected by these authors was higher than that reported in this paper. On the other hand, the yield in ratio of a given oil to medium was lower by 44% and cultivation time had been extended.

The obtained results make it possible to say that the selected yeast strains clearly preferred sources of carbon and energy other than carbohydrates. Similar observations were made by Achremowicz et al. [1]. *Candida lipolytica* and *Candida tropicalis* used in their experiments for propagatien of yeast in n-paraphins yielded twice as much biomass on hydrocarbon medium versus glucose medium.

EFFECTS OF CARBON SOURCE ON PROTEIN YIELD

The effects of carbon source on the protein contents in biomass of the investigated strains and intensity of its synthesis are given in Tables 2 and 3. The highest levels of protein were observed in the biomass of yeast propagated in stickwaters without an additional source of carbon. The stickwaters with added carbohydrates yielded somewhat lower levels of protein in the biomass. The stickwaters supplemented with fish oil contained about 10% less protein (for all strains) than in the case of maximum values obtained from the same stickwaters but protein yield intensity was more than double. According to the literature the contents of protein in the biomass of yeast, depending on strains and substrats, range between 23% and 75%; in most cases the interval is 40% to 50% [11]. In different strains the propagation of yeast in lipids yielded biomass containing between 34% and 49% protein [6, 8, 10]. The figures stand as a good evidence that yields of biomase and protein from stickwaters with added fish-oil were good.

CONCLUSIONS

1. Stickwaters from fish meal production are a good substratum for yeast propagation in spite of relatively divergent chemical compositions and contents of substances impeding the growth of yeast.
2. It was observed that the kind of carbon source had a significant effect on the investigated strains in stickwaters from fish meal production. The most efficient source in this case was fish oil, which is a component of the stickwaters.
3. Propagating yeast in stickwaters can render 23 to 24 dry weight biomass and 10 g protein from one dm^3 substrats containing 0,4% nitrogen and 2% fish oil, over 24 to 30 hrs. This can also reduce the level of organic nitrogen in substratum by ca 40%, and the total contents of organic substances by ca 50%.

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WPŁYW ŹRÓDŁA WĘGLA NA EFEKTYWNOŚĆ ZDROŻDŻOWANIA ODCIEKÓW RYBNYCH

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S t r e s z c z e n i e

Zbadano wpływ źródła węgla na efektywność zdrożdżowania odcieków po produkcji mączki rybnej przez wyselekcjonowane szczepy rodzaju Candida. Podłoże stanowiły odcieki odtłuszczone, odbiałczone i rozcieńczone do zawartości azotu ogólnego 4 mg/cm³ (tabela 1) oraz uzupełnione 0,2% dodatkiem KH_2PO_4. Jako stymulujące wzrost źródła węgla stosowano 3% dodatek węglowodanów w postaci glukozy i melasu lub 2% technicznego oleju rybnego. Na podłożu z odcieków uzyskiwano po 24-36 h hodowli od 7,9 do 8,8 g suchej substancji biomasy z dcm³ przy ok. 20% wykorzystaniu związków azotowych i ok. 30-35% wykorzystaniu suchej substancji podłoża (tabela 2).

Po uzupełnieniu podłoża węglowodanami uzyskano po 70 h hodowli najwydajniejszych szczepów średnio od 20 do 23 g suchej substancji biomasy z dcm³ o zawartości białka od 50 do 53%, z 32-36% wykorzystaniem azotu i około 97% wykorzystaniem cukru (tabela 3).

Najwyższą efektywność zdrożdżowania uzyskano na podłożu z odcieków z dodatkiem 2% technicznego oleju rybnego (tabela 2). Po 24-30 h hodowli otrzymywano średnio 23-24 g suchej substancji biomasy z dcm³ z ok. 40% wykorzystaniem azotu i 50% wykorzystaniem suchej substancji podłoża.