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EFFECT OF MEDIUM PURITY ON SUBMERGED CITRIC ACID FERMENTATION YIELD (WITH PARTICULAR EMPHASIS ON MINERAL SALTS CONTENT)

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The effect of medium purity on submerged citric acid fermentation was studied. It was found that total acidity and, consequently, acid fermentation yield decreases with the increase of ash concentration in the medium. Of all the main ingredients of molasses minerals (potassium, sodium, calcium), calcium ions are the ones inhibiting citric acid fermentation most.

INTRODUCTION

The carbohydrates most frequently used as media for submerged citric acid fermentation with *Aspergillus niger* moulds are sacharose in the form of white sugar, sugar beet and sugar cane molasses, and starch. In the U.S.A. and other sugar cane farming countries, high-test cane syrup is used in addition to sugar cane molasses [1-3]. This syrup, a product made of condensed sugar cane juice, contains about 50% inverted sugar and 20-30% saccharose. A particularly important feature of the inverted syrup, which decides about its applicability in citric acid production, is its low minerals content amounting to 1.2-2.4%. Sugar beet and sugar cane molasses contain much more ash, namely 8-12% [4]. In Poland, a sugar industry product matching high-test syrup in purity is thick juice.

The basic components of molasses minerals are potassium, sodium and calcium. Potassium usually has no adverse effect on fermentation, even when it is present in high concentrations. Sodium compounds, on the other hand, are reported in the literature as playing diverse roles in citric acid fermentation. A positive role, of Na^+ ions is reported by Shoji Usami et al. [12], with this role, however, being more apparent in surface fermentation. Sodium hydroxide is used to partly neutralize wort [6], adjust pH [6, 7] and control mycelium growth in media with saccharose [8] which leads to a ca 5% increase of citric acid yield.

According to Banik [9], only low concentrations of sodium chloride failed to affect citric acid fermentation.

The third element occurring in large quantities in thick juice and molasse is calcium. Some of the Ca^{2+} ions precipitate during juices concentration, and the rest pass on to the molases. Sugar juices and molasses, particularly those from late periods of the sugar campaign, may contain high proportions of Ca^{2+} ions. Experiments with thick juice fermentation demonstrated a decisively negative effect of calcium ions. The adverse effect of these ions on citric acid fermentation is also reported by Nowikowa et. al. [10] and Minc [5]. However, attempts at precipitating calcium with ammonium oxalate and removing the calcium ions together with the precipitate did not give positive effects, since about 20 various microelements precipitated together with calcium, including ones essential for mycelium growth. As a result, when ammonium oxalate was used, it was necessary to adjust the fermentation medium composition by adding the missing microelements.

OBJECTIVES OF STUDY

In earlier experiments [4] with thick sugar juice it was found that the purity of the raw material used has a crucial effect on the course and yield of submerged citric acid fermentation. Accordingly, in this research we decided to determine the effect of medium purity, and especially the effect of minerals content, on submerged citric acid fermentation.

METHODS

Fermentation was carried out in 10 dm^3 fermenters, using selected *A. niger* strains. Fermentation media were made of diluted thick juice and molasses to which suitable salts were added; the composition of the media is given in Table 1.

Table 1. Characteristic of the used molasses and thick juice

Raw material	Dry weight	Sucrose content % weight	Purity quotient	pH	Ash	Ca^{2+}	K^+	Na^+
					mg/100 g dry weight			
Thick juice	52.1	47.8	91.5	8.45	2420	610	1003	88
Molasses	81.6	50.0	63.6	8.15	9500	635	3768	502

Ash content was determined by the weight method after incineration in a platinum crucible. Potassium, sodium and calcium were analysed in a flame photometr. K^+, Na^+ and Ca^{2+} ions were added to wort in the from of chlorides. The course of fermentation was controlled by determinations of total acidity and by micro- and macroscopic observations of the mycelium. The following determinations were made in the postfermentation broth: the quantity of unfermented sugars, mycelium dry weight, citric acid contents, and contents of other acids. Total acidity was determined by titration of 2 cm^3 of fermentation broth with 0.1 n NaOH against phenolphthalein. Reducing sugars were determined with the method of Lane Eynon, and mycelium dry weight after separation of the mycelium from the broth washing, and drying to constant weight in a drier at 105°C. Citric acid was determined as calcium citrate after removal of oxalic acid from the sample. Oxalic acid was determined by high-temperature precipitation of calcium oxalate with calcium chloride which was isolated, dissolved and titrated with 0.1 n $KMnO_4$.

RESULTS

As can be seen in Table 1, the studied molasses are less pure (63.6%) than thick juice (91.5%) and have a higher ash content (9.50% as opposed to 2.4% in thick juice). The effect of medium purity on fermentation yield was thus studied by replacing 10, 20, 30,...% of the molasses nutrient with thick juice in the fermentation medium. The obtained results indicate that increases of the purity quotient lead to proportional increases of total acidity and citric acid yield, and to decreases of oxalic acid and mycelium biomass contents (Fig. 1).

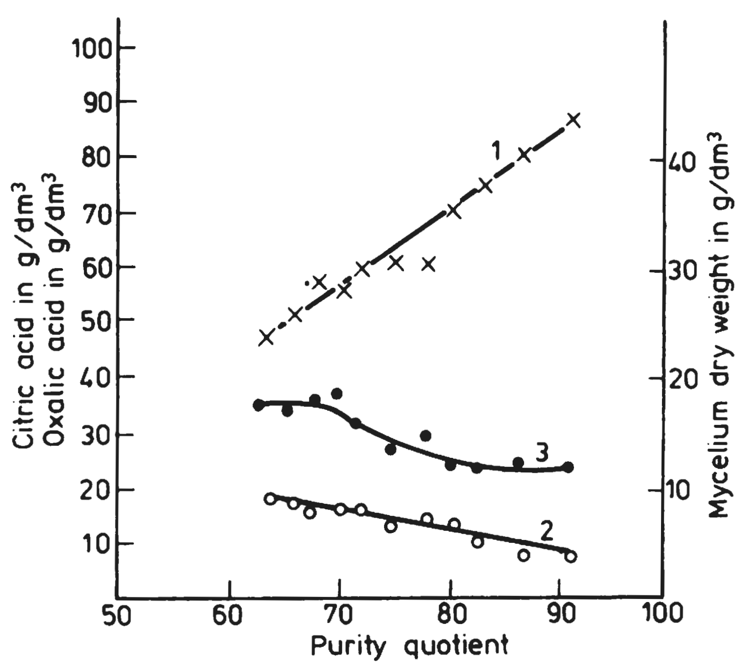

Fig. 1. Effect of medium purity on citric acid fermentation

The highest yields of citric acid obtained for purity quotient 91.5 (thick juice) and the lowest yields for purity quotient 63.6 (molasses). We then proceeded to find out which of the medium components inhibit fermentation. The two studied media differed mainly as to ash content, and we decided to check whether mineral salts are the inhibitory agents. A molasses sample was mineralized and then added to the thick juice medium in increasing amounts until its content reached

19.1 g/dm^3 of wort, i.e. the content corresponding to the minerals amount in a molasses medium with the same sugars concentration. The initial pH of the medium was adjusted separately for each molasses addition with excess hydrochloric acid after reaction with the first portion with oxides of the elements introduced in the form of ash. The results of this fermentation (Fig. 2) show clearly that an increases of minerals content leads to a proportional drop in citric acid yield, and to an increase in mycelium weight. The excessive growth of the mycelium also caused a considerable reduction of citric acid yield. Ash additions in excess of 2 g/dm^3 of the medium eliminated oxalic acid from the postfermentation solution: in such conditions this acid precipitated in the form of calcium salt and was removed together with the mycelium. It was thus estabished that minerals is among the principal factors inhibiting submerged fermentation of citric acid. In subsequent experiments we tried to single out the ash component which inhibits fermentation most.

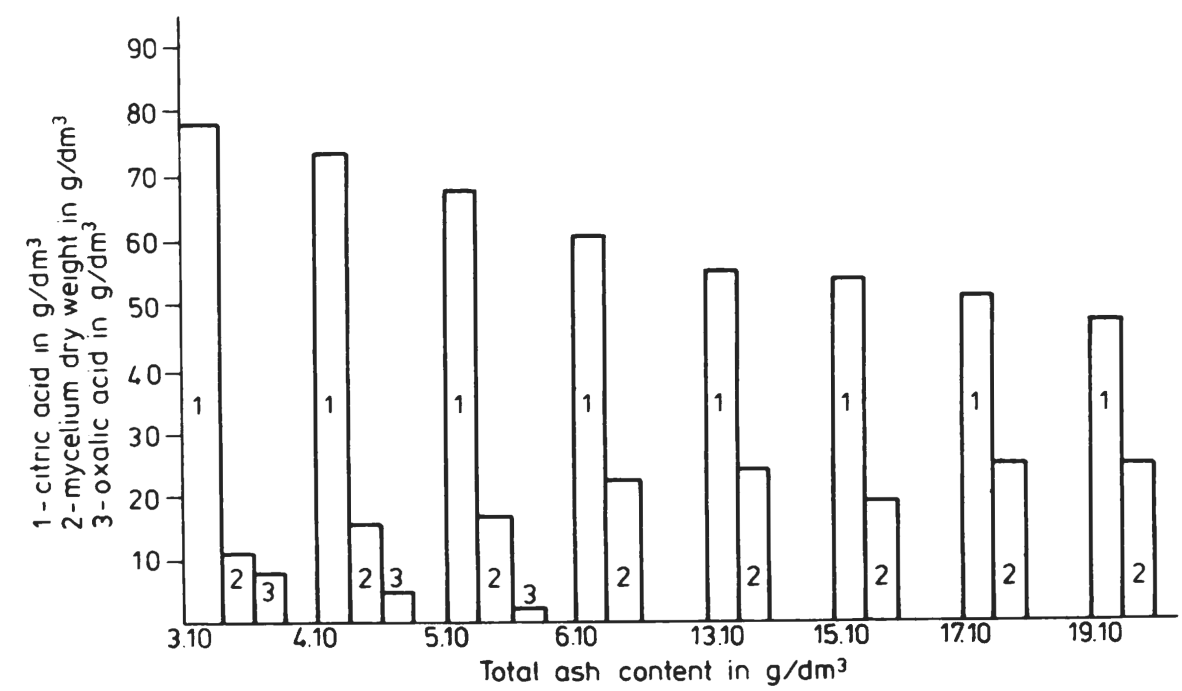

Fig. 2. Effect of molasses minerals concentration on citric acid fermentation

The cations most plentiful in thick juice and molasses are K^+ ions which may outnumber Na^+ and Ca^{2+} ions several times. Since potassium content in molasses varies considerably, its effect was determined at concentrations ranging from 1.5 to 8.0 g/dm^3 (Fig. 3). No inhibitory effect of K^+ ions was observed, citric acid yield was usually unchanged, and additions of K^+ ions did not lead to the formation of other acids.

Next we examined the effect of sodium ions. Similarly as in the case of K^+ ions, the amounts of Na^+ ions may vary greatly in sugar juices and molasses. In our experiments we applied doses exceeding 2.5 times the sodium ions concentration in media prepared from the studied molasses. The results (Fig. 4) show that the highest dose of Na^+ ions (2.5 g/dm^3) reduces citric acid yield by 13% (from 69.3 to 56.5%). The postfermentation broth (excepting control) did not contain oxalic acid, this being due to the fact that the small quantities of this

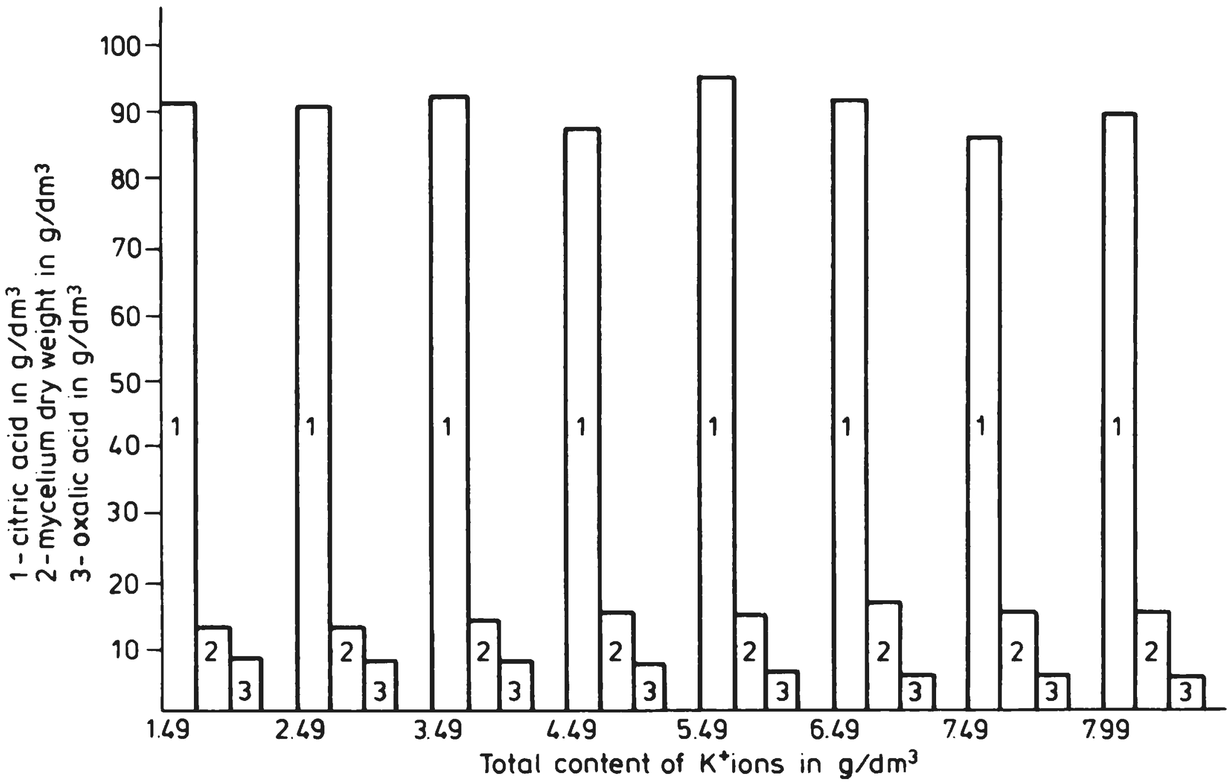

Fig. 3. Effect of K^+ ions on citric acid fermentation

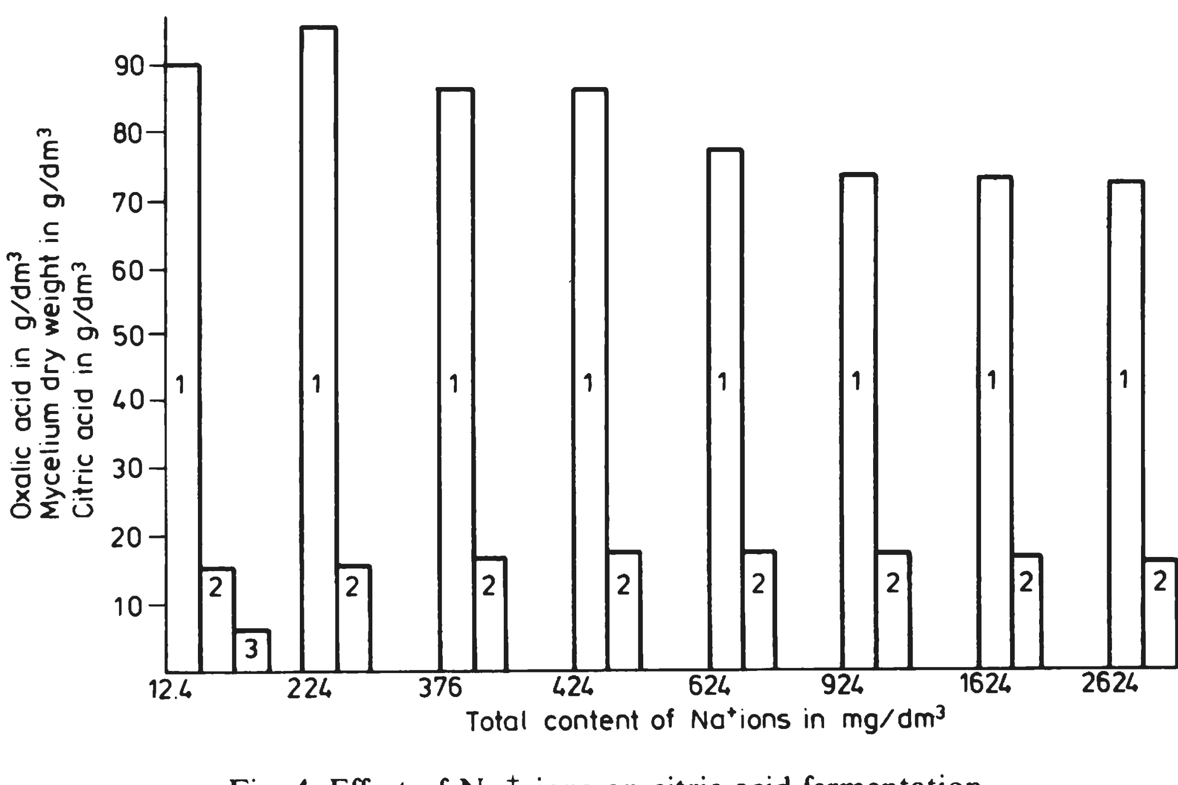

Fig. 4. Effect of Na^+ ions on citric acid fermentation

acid that appear, from with the introduced Na^+ ions the poorly soluble sodium oxalate which is removed together with the mycelium. This indicates that excessive amounts of sodium ions in the medium inhibit citric acid fermentation.

The Ca^{2+} ions additions in our experiments ranged from 0.5 to 3.0 g/dm^3. We found that these ions inhibit citric acid formation to a greater extent than Na^+ ions (Fig. 5). There are visible drops in citric acid amounts with every addition of calcium. All doses extended fermentation by about four days, and doses over 1.0 g/dm^3 caused incomplete fermentation of sugar. Mycelium dry weight increased by about 15% already at the 0.5 g/dm^3 dose, but then remained constant when

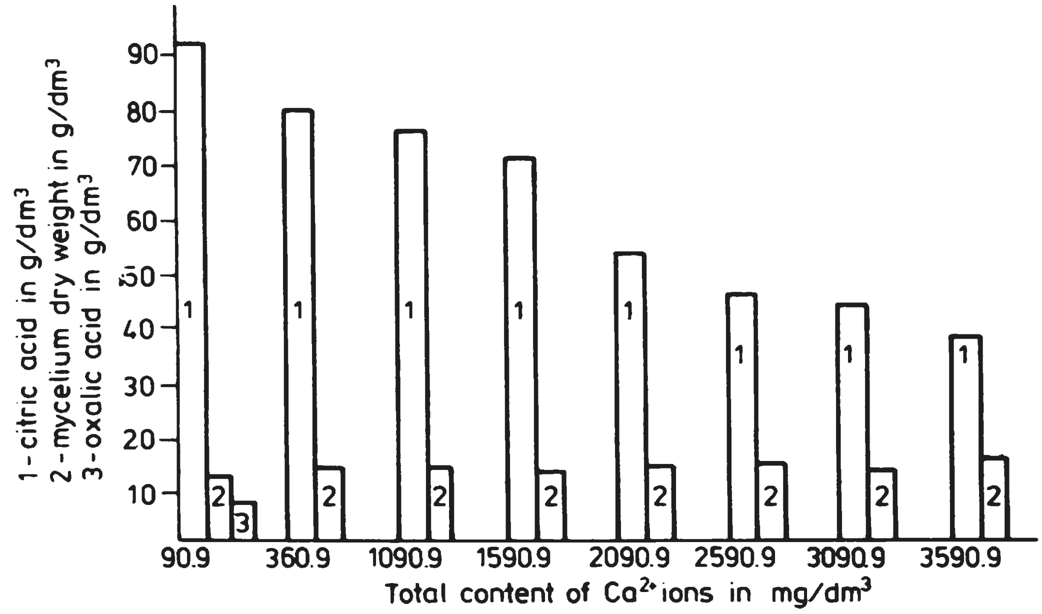

Fig. 5. Effect of Ca^{2+} ions on citric acid fermentation

more calcium was added to the medium. This means that greater biomass increment was not the only factor responsible for the drop in citric acid yield. Similarly as in experiments with the Na^{+} ions, there is no oxalic acid in the postfermentation broths it being removed together with the mycelium in the form of calcium oxalate.

Subsequent experiments were meant to confirm the strongest inhibitory effect of Ca^{2+} ions. Wort made from thick juice was treated with incinerated molasses which were previously deprived of Ca^{2+} ions by means of oxalic acid. The obtained results (Fig. 6) indeed demonstrate that calcium ions are the principal

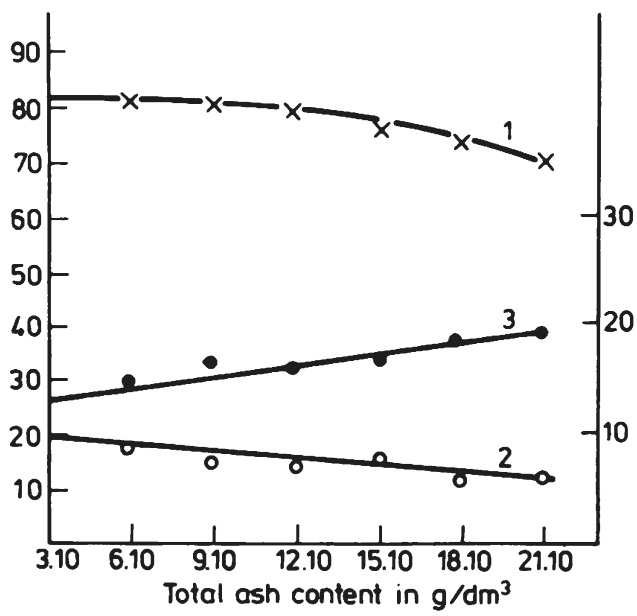

Fig. 6. Effect of molasses minerals without Ca^{2+} ions on citric acid fermentations

components of ash responsible for the inhibition of citric acid fermentation, since the experimental ash without these ions affected the formation of citric acid to a much smaller extent. For the ash dose of 18 g/dm^3, citric acid yield was only 7% lower, and the effect of smaller doses was barely perceptible.

In further experiments we fermented media made from molasses obtained

Table 2. Citric acid fermentation yields in molasses from different sugar factories

Molasses from factory	Composition of molasses				Fermentation yield for strains			
	dry weight %	Ca^{2+}	Na^{+}	K^{+}	*A. niger* B-64-5		*A. niger* I-13	
		mg/100 g dry weight			acid yield %	mycelium dry weight g/dm^3	acid yield %	mycelium dry weight g/dm^3
Cerekiew	80.4	311	357	5224	57.8	17.3	55.1	20.3
Wieluń	80.8	433	495	4332	52.4	18.7	50.8	20.2
Chybie	80.0	437	1000	3375	48.7	12.7	48.6	16.3
Racibórz	79.0	443	443	5063	49.3	12.9	48.1	14.0
Baborów	80.0	500	500	5000	51.3	17.0	54.0	19.3
Częstocice	78.0	705	256	3205	42.1	15.6	37.5	16.1
Werbkowice	82.4	727	486	4247	42.7	16.3	42.0	18.7
Otmuchów	79.2	758	316	4420	59.3	19.0	49.9	13.3
Włostów	83.6	897	239	2632	35.0	17.7	35.4	23.6
Wróblin	81.0	1237	247	4320	24.0	15.0	23.0	20.6

from ten different sugar factories, and hence having various chemical composition. Potassium, sodium and calcium were determined in all the molasses, which were then fermented with two strains of *A. niger*, and arranged in the order of increasing calcium ions content which ranged from 311 to 1237 mg/100 g dry weight (Table 2). The greatest citric acid yields were obtained in the case of molasses from the "Cerekiew", "Wieluń" and "Baborów" factories, containing, respectively, 311, 433 and 500 mg Ca^{2+} ions in every 100 g dry weight of molasses. These particular molasses had relatively large amounts of K^+ ions: over 5000 mg/100 g dry weight ("Baborów", "Cerekiew") and 4332 mg/100 g dry weight ("Wieluń"). The higest Na^+ ions content, 1000 mg/100 g dry weight, was in molasses from the "Chybie" sugar plant which gave a moderate fermentation yield. The highest Ca^{2+} ions contents were in molasses from "Wróblin" and "Włostów" (1237 and 897 mg/100 g dry weight, respectively), and these had a very poor yield of citric acid fermentations with both *Aspergillus niger strains.* All this confirms that calcium ions present in fermentation media inhibit the biosynthesis of citric acid.

CONCLUSIONS

1. The effectiveness of submerged citric acid fermentation depends to a large degree on medium purity, and especially on the amounts of mineral substances in the medium. Decreasing purity of the medium and increases in mineral substances content decrease citric acid yield.

2. Ca^{2+} ions are among the most harmful substances contained in molasses ash.

3. When natural juices are not alkalic — and this happens most often in the initial and final stages of the sugar campaign — it is difficult to remove their calcium salts during purification. As a consequence, large amounts of these salts survive the various production phases and end up in the molasses which, having excessive quantities of Ca^{2+} ions, are not suitable for citric acid fermentation.

4. The drop in natural alkalinity of sugar juices is being artificially compensated by additions of Na^+ ions in the from of sodium carbonate or sodium hydroxide. This increases the Na^+ ions content in juices and molasses which should not be used in the production of citric acid. Molasses of this kind are obtained from unripe or technologically degraded sugar beet. One should also avoid producing citric acid from molasses which had their pH increased with sodium carbonate or sodium hydroxide.

5. The experiments also revealed that large amounts of K^+ ions have no adverse effect on citric acid fermentation.

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BADANIA NAD WPŁYWEM CZYSTOŚCI PODŁOŻA, A ZWŁASZCZA SOLI MINERALNYCH NA CYTRYNOWĄ FERMENTACJĘ WGŁĘBNĄ

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

Badano korelacje występujące pomiędzy czystością podłoża a efektywnością wgłębnej fermentacji cytrynowej. Badania prowadzono w skali laboratoryjnej w fermentorach o pojemności 10 dm^3 przy użyciu wybranych szczepów *A. niger*. Jako główny substrat stosowano melasę buraczaną i cukrowniczy sok gęsty oraz ich mieszaniny. Stwierdzono, że wraz ze zmniejszeniem się czystości podłoża maleje wydajność fermentacji cytrynowej (rys. 1). Głównym powodem zmniejszenia się wydajności na tych podłożach była większa zawartość soli mineralnych. Zostało to potwierdzone w próbach fermentacyjnych z rosnącym dodatkiem popiołu melasowego (rys. 2). Spośród głównych składników popiołu: potasu, sodu i wapnia nie stwierdzono ujemnego wpływu nawet dużych ilości, jakie występują w melasie potasu (rys. 3). Sód w ilościach ponad 300 mg/dm^3 wpływał już wyraźnie ujemnie (rys. 4) podobnie jak wapń (rys. 5), który był największym inhibitorem fermentacji. Ujemny wpływ wapnia został potwierdzony w próbach fermentacyjnych z dodatkiem popiołu melasowego, z którego usunięto jony wapnia (rys. 6) oraz w próbach fermentacyjnych prowadzonych na melasach pochodzących z różnych cukrowni, a różniących się zdecydowanie zawartością wapnia (tab. 2), w których najwyższe wydajności uzyskano w próbach zawierających najmniejsze ilości wapnia.