The Influence of some trace-elements on the Growth of Aspergillus niger and on the citric acid Fermentation

By S. P. Tandon and A. S. Srivastava

(Chemistry department, University of Allahabad, Allahabad, India)

Introduction

Steinberg (1935) and Lund (1953) found that the growth of *Aspergillus niger* is influenced by copper. Orlov et al. (1968) have further observed that 10.0% solution of copper sulphate increases markedly the yield of citric acid when molasses is employed as substrate. Tomlinson et al (1950) studied the influence of Zn, Fe, Mn and Cu on the production of citric acid and observed that zinc and iron are essential trace-elements for the maximum yield of citric acid. The results of study relating to the influence of trace-elements on the production of citric acid are apparently conflicting. Whit some samples of *A. niger*, Erkama and co-workers (1949) observed that iron and zinc accelerate the formation of citric acid, while with other samples of *A. niger* iron and zinc do not appear to be favourable for the good yield of citric acid. This contradictory finding in citric acid fermentation has been ascribed to the difference in strains of *A. niger*. This finding was also supported by Quilco and Dicapua (1932). Perlman (1946) observed that the optimum concentration of a particular trace-element changes with the strain of *A. niger* selected. He noted that the addition of 0.10 mg. of iron per litre to one medium was optimum for the yield of citric acid in the case of *A niger 62*, while a concentration of 1.0 mg. of iron per litre was optimum for *A. niger 69, 70 and 72*. It was also reported that molybdenum, copper and zinc inhibited the production of citric acid. Wataru (1966) observed that the addition of 0.5% H_3BO_3 gave good yield of citric acid.

As a result of such interesting and conflicting observations, the role of trace-elements in the growth and activity of the micro-organisms has been the subject of much investigation. The present study deals with an investigation made to evaluate the citric acid producing power of four trace-elements (Lanthanum sulphate, Zirconium sulphate, Praseodymium sulphate and Uranium sulphate).

Experimental

150.0 g of sucrose, 2.5 g NH_4NO_3, 1.0 g KH_2PO_4 and 0.25 g $MgSO_4 \cdot H_2O$ were dissolved in 500 ml distilled water and a requisite amount of

KCl-HCl buffer solution was added to the medium to adjust pH 2.2. The total volume of the medium was divided into 10 equal parts and each part was taken in a 250 ml conical flask. In this way forty eight flasks containing the above quantity of the medium were arranged for the experiment with each trace-element taken for study. These flasks were arranged in three sets, each comprising of 15 flasks. The remaining three flasks were kept as controls. Each set was re-arranged in five sub-sets, each comprising of 3 flasks. These five sub-sets of each experiment contained 1, 2, 3, 4 and 5 ml of N/1000 solution of the respective trace-element. Thus, the molar concentration of the trace-element was 1.0×10^{-5} M, 2.0×10^{-5} M, 3.0×10^{-5} M, 4.0×10^{-5} M and 5.0×10^{-5} M.

The control flasks did not contain any trace-element. Finally, the volume of the medium in all the flasks was raised to 100 ml. Then the flasks were plugged with cotton and sterilized at 15 lb. pressure for thirty minutes. After sterilization they were allowed to cool to room temperature.

A heavy spore suspension was obtained by pouring 10 ml of sterilized distilled water over a young colony (3 days old) of *A. niger strain 21* grown in a culture tube. The tube was shaken to ensure maximum dispersion of the spores. Three drops of this spore suspension were added to each flask. The flask were then inoculated at 30° C in an incubator.All the fifteen flasks of each of the three sets were analysed after 5, 10 and 15 days of incubation respectively.

The mold was separated from the medium by filtration through a weighed filter paper. The mycelium mat of the mold was washed three or four times with distilled water and dried in an air oven at 60—70° C for 24 hours and then cooled in a desiccator and weighed. Thus, the amount of the growth of the mold in terms of dry weight was obtained.

Citric acid was determined gravimetrically as the calcium salt. The filtrate along with washings was collected and the total volume was made upto 100 ml by adding requisite amount of distilled water (Sol. A).

10 ml of the above solution was taken and it was hydrolyzed with 2 ml of 2 N HCl by boiling for 10 minutes. After cooling the excess of the acid was neutralized by a few drops of dil. sodium hydroxide. The solution was then made upto 100 ml and was titrated against standard Fehling solution using methylene blue as an indicator (Lane-Eynon 1924).

Now 50 ml of the sol. A was taken in a 250 ml beaker and an excess of ammonia was added to neutralise the acid. The solution was boiled till the excess of ammonia was removed. Then 2% $CaCl_2$ solution was added to precipitate both oxalic and citric acids as calcium oxalate and calcium citrate. After cooling the ppt. was filtered through a weighed filter paper, washed three or four times with destilled water and was dried. The ppt. was weighed and was then treated with hot 50% acetic acid. The calcium oxalate remained on filter paper, while calcium citrate dissolved in acetic acid. The ppt. of calcium oxalate was reweighed and, thus, the amount of calcium citrate was obtained by difference. From the amount of calcium citrate obtained the amount of citric acid could be known.

Observation

Table 1

Influence of Lanthanum sulphate on the growth of the mold and on the production of citric acid

Conc. of Lanth. sulphate	Growth of the Mold in g./100 ml			Yield of Citric Acid in g./100 ml			Sugar left in g./100 ml		
$a \times 10^{-5}$ M	Days 5	10	15	5	10	15	5	10	15
1.0	0.7020	1.5992	2.1820	0.5040	1.8345	2.6812	12.022	7.020	4.450
2.0	0.4022	1.6802	2.2162	0.5022	1.3582	3.1202	11.842	6.420	6.068
3.0	0.3262	1.6722	2.500	0.4024	1.5600	3.6230	12.000	5.752	4.910
4.0	0.00	0.00	0.00	0.00	0.00	0.00	13.580	12.040	11.02
5.0	0.00	0.00	0.00	0.00	0.00	0.00	13.472	11.642	11.00
Control	1.6284	2.6860	3.3884	1.2622	3.3170	4.9236	12.660	7.542	4.324

Table 2

Influence of Zirconium sulphate on the growth of the mold and on the production of citric acid

Conc. of Zirconium sulphate	Growth of the Mold in g./100 ml			Yield of Citric Acid in g./100 ml			Sugar left in g./100 ml		
$a \times 10^{-5}$ M	Days 5	10	15	5	10	15	5	10	15
1.0	0.400	1.0260	1.5050	0.3004	0.5542	3.0086	13.440	10.02	6.546
2.0	0.4830	1.3980	2.0902	0.4400	0.8580	4.2592	13.438	8.402	5.462
3.0	0.6262	2.2200	2.2860	0.6042	1.0204	4.2680	12.862	8.006	4.860
4.0	0.6002	1.4522	2.030	0.600	1.0686	4.4626	12.850	7.545	3.540
5.0	0.00	0.00	0.00	0.00	0.00	0.00	13.00	12.068	11.04
Control	1.7240	2.8120	3.2862	1.062	3.3500	4.8804	13.00	7.600	4.600

Table 3

Influence of Praseodymium sulphate on the growth of the mold and on the yield of citric acid

Conc. of Praseodymium sulphate	Growth of the Mold in g./100 ml			Yield of Citric Acid in g./100 ml			Sugar left in g./100 ml		
$a \times 10^{-5}$ M	Days 5	10	15	5	10	15	5	10	15
1.0	0.5222	1.3660	2.0802	0.6624	1.7818	2.0974	13.020	9.930	6.752
2.0	0.5268	1.5080	2.400	0.7864	2.0442	2.5742	12.862	9.440	5.850
3.0	0.7860	1.9642	2.8824	0.8606	2.8256	3.7232	13.024	8.740	4.505
4.0	0.00	1.4020	2.5200	0.00	1.8402	3.2572	14.00	9.332	4.826
5.0	0.00	0.00	1.6640	0.00	0.00	2.5400	14.04	12.680	5.800
Control	1.800	2.6850	3.1784	1.1864	3.4082	4.8634	12.340	7.546	4.232

Table 4

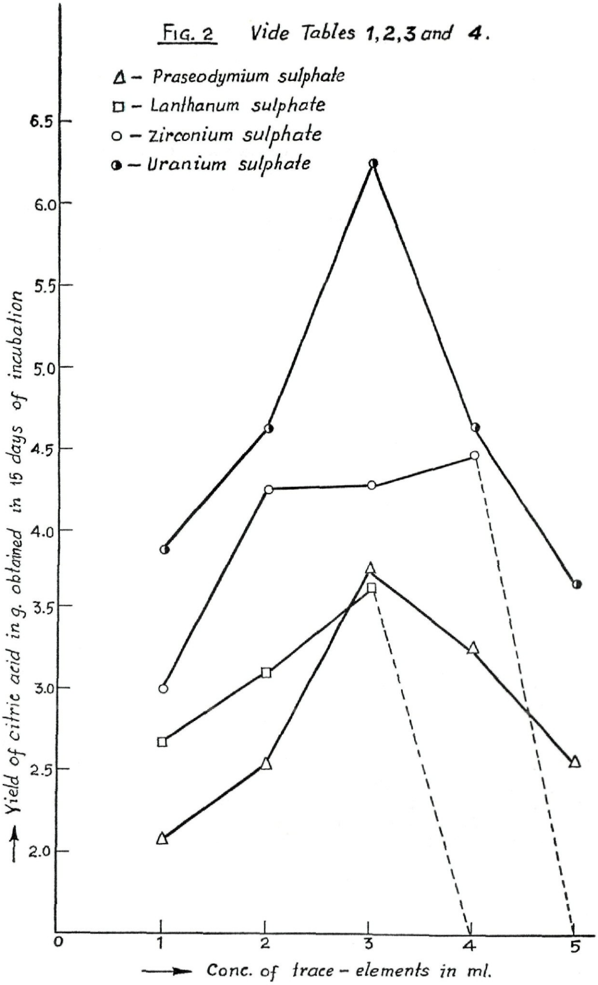
Influence of Uranium sulphate on the growth of the mold and on the yield of critic acid

Conc. of Uranium sulphate	Growth of the Mold in g./100 ml			Yield of Citric Acid in g./100 ml			Sugar left in g./100 ml		
$a \times 10^{-5}$ M	Days 5	10	15	5	10	15	5	10	15
1.0	2.0022	2.4720	3.3200	0.7024	2.1040	3.8562	13.822	11.400	7.02
2.0	2.2826	2.9220	3.802	0.9602	2.6002	4.6242	13.320	11.240	6.54
3.0	2.6642	3.2200	4.0100	1.3102	3.4316	6.2630	12.044	10.652	5.80
4.0	2.0704	2.8040	3.800	0.990	2.800	4.6240	12.820	10.962	6.400
5.0	1.8340	2.4002	3.2204	0.6642	1.8212	3.6752	13.00	11.00	7.01
Control	1.7452	2.6502	3.2420	1.1204	3.3242	4.9422	12.550	6.802	4.200

Discussion

The data given in table 1 show that the influence of lanthanum sulphate was insignificant. It was observed that even the maximum growth of the mold as well as the maximum yield of citric acid in all 5, 10 and 15 days of incubation period were comparetively less than that in the control. It was, however, observed that the lower concentrations of lanthanaum sulphate were favourable for the maximum activity of *A. niger,* while higher concentrations proved to be inhibitory and at more higher concentration of lanthanum sulphate (4.0×10^{-5} M and 5.0×10^{-5} M) there was neither any amount of growth nor there was any amount of citric acid produced. Although, the influence of zirconium sulphate (as recorded in table 2) was also found to be insignificant, yet its influence in producing citric acid was slightly encouraging. It appears that the influence of these trace-elements might be deactivating the enzyme system involved in citric acid fermentation which causes poor yield of citric acid. The influence of

FIG. 2 Vide Tables 1, 2, 3 and 4.



another trace-element, praedymium sulphate (as recorded in table 3) both on the growth of the mold as well as on the yield of citric acid also remained the same. The maximum growth of the mold as well as the maximum yield of citric acid both were found to be less than that of the control.

So far the influence of Uranium sulphate (as recorded in table 4) was concerned, it was found to be significant. It was evident from the fact that even the minimum growth of the mold as well as the minimum yield of citric acid were comparatively better than the maximum growth of the mold and the maximum yield of citric acid obtained with the influence of lanthanum sulphate, zirconium sulphate and praseodymium sulphate.

With all the four trace-elements studied in the present investigation it was observed that usually the consumption of sugar during the course of fermentation corresponded with the production of citric acid.

Summary

While summarising the influence of all the trace-elements studied in the present investigation (vide graph 1 and 2) it was concluded that the influence of lanthanum sulphate, zirconium sulphate and praseodymium sulphate on the production of citric acid was found to be insignificant, while that of uranium sulphate could produce influence upto a great extent. In its presence the highest percentage of citric acid obtained was 68.2, where as in the case of lanthanum suphate, zirconium sulphate and praseodymium suphate these were 35.9%, 39.2% and 45.0% respectively.

References

1. Wooley, D. W., J. Biol. Chem., **140**, 311—12 (1941).
2. Steinberg, R. A., Bull Torrey Bot. Club, **62**, 81 (1935).
3. Lund, N. A., J. Chem. Soc., 2434 (1953).
4. Orlov, I. N., Fedossev, V. F. Lyubarskii, A. F. and Rudcheuko, A. K., Izo bret., Prom. Obraztsy Tovaruya Znaki., **45** (33), 20 (1968).
5. Tomlinson, N., Campbell, J. S. R. and Trussel, P. C., J. Bact., **59**, 217—27 (1950).
6. Erkama, J., Hagerstrand, H. B. and Junkkonen, S., Acta. Chem. Scand., **3**, 862—6 (1949).
7. Ouilico, A. and Di capua, A., Chemica, e. Industria, **14**, 289 (1932).
8. Perlman, D., J. Bact., **49**, 167—175 (1946).
9. Wataru, T., Hakko Kyokaishi, **24** (8), 339—64 (1966).

Zeitschrift/Journal: Sydowia

Jahr/Year: 1971/1972

Band/Volume: 25

Autor(en)/Author(s): Tandon S. P., Srivastava A. S.

Artikel/Article: The Influence of some trace-elements on the Growth of Aspergillus niger and on the citric acid Fermentation. 137-142