Tetsu SAKAMURA and Kimiko MAEDA: On the assimilation of
nitrate-nitrogen by *Hansenula anomala*.

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Received 19. May, 1949

The reduction of nitrate by *Hansenula anomala* which had previously been cultured with addition of molybdenum in minute concentration (1×10^{-6} M), took place more slowly than without it. Nitrate accumulated as a reduction product in the nitrate culture with molybdenum, while it was not at all or scarcely detected in the $-Mo^*$ culture.

pH	+Mo > -Mo	Molybdenum limited the consumption of glucose which was needed by the nitrate reduction and this restrictive action favored rather the growth of the yeast and it resulted in the increase of the economical coefficient. Molybdenum can be regarded as a regulator which limits the waste of free energy in the metabolism in this meaning. Such an action of molybdenum has not been observed in the culture with ammonium salt. The nitrate reduction occurred more rapidly in the aerobic condition than the anaerobic. No re-oxidation of the reduction products happened.
Accumulation of NO_2^-	+Mo > -Mo	
Accumulation of NH_3	+Mo > -Mo	
Accumulation of amide	+Mo < -Mo	
Consumption of NO_3^-	+Mo < -Mo	
Consumption of glucose	+Mo < -Mo	
Growth (NO_3^- -culture)	+Mo > -Mo	
Growth (NH_3 -culture)	+Mo = -Mo	
Economical coefficient	+Mo > -Mo	
$NO_3^- \rightarrow NO_2^-$	+Mo < -Mo	
$NO_2^- \rightarrow NH_3$	+Mo < -Mo	
Glucose-dehydrogenation:		
NO_3^- -culture	+Mo < -Mo	
NH_3 -culture	+Mo = -Mo	

Among the partial reactions of the nitrate reduction, the dehydrogenation by the yeast cultured without molybdenum was more active than the yeast with it, but no influence of this metal appeared on the reductase system. Between the cultures with and without molybdenum, supplied with nitrate or ammonium salt as nitrogen source, there was a remarkable difference in accumulation of amide and ammonia produced from these nitrogen sources. From the results obtained in the present investigation it may be concluded that there is a certain oxidative reaction after the synthesis of amino acid, which has a far reaching influence on the velocity of the reduction of nitrate and nitrite. As such an oxidative reaction we may presumably consider a reaction connected with the formation of amide which is regarded to need a supply of free oxygen in several living materials. The above mentioned action of molybdenum seems to occur on this assumed reaction, but not on the reductase reaction itself, though the dehydrogenase system is not indifferent to the action of this metal.

The higher the acidity, the more intensely the reductase exceeded the dehydrogenase in the action, and *vice versa*. Urethane did not inhibit the nitrate reduction itself, but had an influence on the dehydrogenation. When glucose was used as the substrate, the reductive reaction catalysed by nitrate reductase was retarded by moniodo-acetic acid, while the reaction depending on nitrate reductase was insensitive to it. Cyanide inhibited the reduction both of nitrate and nitrite, as demonstrated in bacteria by many authors. (Department of Botany, Faculty of Science, Hokkaido University, Sapporo).

* $-Mo$: Culture without molybdenum. $+Mo$: Culture with molybdenum.