INFLUENCE OF AERATION LEVELS ON NEOMYCIN TITRE IN CONTINUOUS STIRRED REACTOR BY *STREPTOMYCES MARINENSIS*

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SUBMERGED fermentations are almost universal in the development of new antibiotics and other industrial fermentations. The provision of an adequate air supply is essential for metabolic and synthetic activities of the micro-organism. Bartholomew *et al*[1] measured the rate of oxygen diffusion in the presence and absence of agitation to correlate the product formation. They determined the yields of penicillin and streptomycin from broths under different conditions of aeration and found that titres increased with aeration, up to a certain point where the rate of solution of oxygen satisfied the rate of demand of the culture. Various investigators[2–7] studied the relation between oxygen transfer rate and product formation. Markkanen and Bailey[8] studied the effect of varying aeration levels on the production of α-amylase and got maximum yield at low VVM.

Micro-organisms require greater aeration during the growth phase but their requirement of air during stationary phase is smaller. The cost of aeration in submerged fermentations is high. Trials can be made to bring down the production cost by reducing aeration levels at any appropriate stage. The aim of the present work is to get the maximum antibiotic titre with minimum aeration levels.

The organism used in this work was a new streptomycete, *Streptomyces marinensis*[9]. It was subcultured from the stock on jowar starch agar containing 2.5% jowar starch, 1% corn steep liquor, 0.5% ammonium sulphate, 0.5% sodium chloride, 0.5% calcium carbonate, 1.5% agar, pH 6.5 and incubated for 9 days at 28°C. To prepare the inoculum, the contents of well grown slant cultures were transferred to 100 ml inoculation medium (composition 2.5% soluble starch, 1% corn steep liquor, 0.5% ammonium sulphate, 0.5% calcium carbonate and pH 7.0) and incubated for 48 hr at 28°C on a rotary shaker.

The production medium contained 9% dextrin, 2% soyabean meal, 1% corn steep liquor, 1% sesame meal, 1% calcium carbonate and 1% ammonium sulphate with pH 8.2. Emenvee laboratory fermentor (Emenvee) with 10 lit fermentor jar was used (working capacity of jar 5 l). The inoculum (500 ml) was transferred to the production medium and fermentation carried out at 28°C (aeration at 1 VVM level and speed 500 rpm). Samples were collected at regular intervals up to 192 hr. The packed cell volume was determined by centrifuging the sample in a graduated centrifuge tube at 2000 rpm for 15 min. The antibiotic content was assayed by standard cup-plate method[10,11] using *Bacillus pumilus* NCIM 8982 as test organism. The first set of experiments were conducted with 1 VVM aeration up to 192 hr. In the next series of five experiments 1 VVM aeration was used up to 72 hr (up to log phase) and later 0.75, 0.5, 0.25, 0.1 and 0.0 VVM were used respectively up to 192 hr. The results are given in table 1. During these experiments, one control flask (100 ml of the medium in 500 ml flask) was run on rotary shaker at 220 rpm.

The growth and production data indicated that the stationary phase commenced at 72 hr with simultaneous antibiotic production. Maximum anti-

Table 1 *Effect of varying aeration levels on neomycin titre*

Fermentation time (hr)	Titre in units per ml					
	Aeration in VVM	1.0*	0.75	0.5	0.25	0.1	0.0
72		3500	3440	3480	3500	3520	3400
96		5080	5040	5440	5440	5440	5040
120		8000	8240	8400	9200	6240	3520
144		7200	6800	6720	7840	4240	2880
168		6800	6240	6560	7600	3280	2040
192		5400	5220	4740	5000	–	–

Note: In all experiments aeration level of 1 VVM was maintained during the first 3 days.

biotic titre was obtained at 120 hr. The results of various aeration levels (table 1) showed that maximum antibiotic level was obtained with 0.25 VVM aeration at 120 hr.

The control flask (shake flask on rotary shaker) always gave the maximum yield of 7200 units/ml at 144 hr.

The above data indicate that the provision of adequate aeration increased the antibiotic titre in the fermentor and maximum titre obtained in a shorter period when compared to the shake flasks. Although the difference in the antibiotic titre run at 0.5, 0.75 and 1.0 VVM aeration levels was not significant, the antibiotic yield was maximum at 0.25 VVM aeration level. The data at 0.25 VVM indicates that reduction of air supply towards the end of the growth phase does not significantly affect neomycin titre. Further, it indicates that 0.25 VVM may be the critical level.

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NITROGEN FIXATION AND TRANSFER OF FIXED NITROGEN TO ASSOCIATED CEREAL IN A MAIZE-COWPEA MIXED CROPPING SYSTEM

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In mixed cropping systems there are a number of possible pathways by which nitrogen enters the cereal and legume components of the cereal-legume association. The contribution from soil and fertilizer is directly evaluated by labelling soil or fertilizer N. The contribution from atmospheric source in the case of legume and legume fixed source in the case of cereal is extremely difficult to estimate by using simple isotopic techniques. The use of ^{15}N enriched materials to label the organic N in soil provides a method to estimate the amount of N fixed by a legume. This technique depends on the use of ^{15}N enriched materials incorporated into the soil and pre-incubation of the soil to immobilize the ^{15}N, thus bringing a rapid equilibration of the added N availability ratio. Several workers have compared this technique with other methods. Good agreement was observed between ^{15}N dilution[1] and difference methods[2] but estimates of acetylene reduction method[3] was lower. In the present investigation soil and fertilizer N labelling technique[4] has been adopted for a quantitative estimation of the N fixed by cowpea and transferred to companion maize in a mixed cropping system.

A field study was conducted at IARI, New Delhi in 1981–82. The soil of the experimental site had a pH 8.6, organic C, 0.64%, total N, 0.07%, CEC, 8.2 me/100 g soil and the texture sandy loam. Available P and exchange K content were 0.07% and 1.8 me/100 g soil respectively. The soil belonged to Mehrauli series and had been classified as non-acid hypothermic family of Ustochrepts.

Maize (*Zea mays* L) and cowpea (*Vigna sinensis* L) were grown together and separately to assess the amount of N fixed by cowpea and possible transfer of N to the companion maize. Crops were grown in three combinations viz (i) maize alone, (ii) maize-cowpea mixed cropping and (iii) cowpea alone. The mixed cropping had the same maize plant population as was in pure maize and cowpea was grown in