YIELD DEPRESSION IN METHANOL-UTILIZING
BATCH CULTURES

G. Hamer*, H. S. Pal and I. Y. Hamdan,
Kuwait Institute for Scientific Research,
P. O. Box 12009, Kuwait.

SUMMARY
Yield depression, as opposed to growth inhibition, in batch cultures of methanol-utilizing microorganisms is discussed. Under conditions where the yield coefficient varies, the effect on oxygen demand has been predicted for exponentially growing cultures.

INTRODUCTION
During the past decade considerable interest in the growth of lower alcohol-utilizing micro-organisms has developed. Both methanol and ethanol are used as carbon feedstocks for single-cell protein (SCP) production. Lower alcohols are generally classified as inhibitory substrates, even to those micro-organisms that are able to utilize them as their sole carbon energy sources. For the commercial-scale production of SCP, economic criteria dictate that continuous flow operation of the fermenter is essential. However, for the study of lower alcohol-utilizing micro-organisms in the laboratory, batch culture techniques are frequently used, particularly for the initial screening of culture performance characteristics prior to detailed investigation of the more promising cultures in chemostats.

In methanol-limited chemostat cultures, the micro-organisms are subjected to only small fluctuations in methanol concentration (Harrison and Topiwala, 1974), but in batch cultures, they are subjected to major changes in methanol concentration, depending on the technique used for methanol addition. The most commonly used technique is to add all the methanol prior to inoculation, but sometimes, periodic additions are made during
growth. Methanol can also be added to batch cultures continuously as a vapour mixed with air (Hamer, 1968), and it is possible, although not desirable, to achieve a gradual increase in the liquid-phase methanol concentration as growth proceeds, with this technique.

Two types of inhibition that commonly occur in methanol-utilizing cultures are where the methanol concentration affects the growth rate and where the methanol concentration alters the yield coefficient independently of the growth rate. It is this latter situation that will be examined, in more detail, in this contribution.

**YIELD DEPRESSION OBSERVATIONS**

For SCP production from methanol, any reduction in the yield coefficient is economically disadvantageous. Harrison (1976) suggested that methanol caused uncoupling of either the energy-production or the energy-utilization process when present in excess, suggesting that in methanol-utilizing micro-organisms, little feed-back regulation of the assimilative and oxidative pathways exists. When excess methanol is present, methanol-utilizing micro-organisms oxidize it to carbon dioxide rather than convert it into either intracellular storage or extracellular products. Carbon dioxide production is also accompanied by undesirable, additional heat production.

The basis for this hypothesis was data published by Harrison et al. (1972) concerning the effect of initial methanol concentration on the yield coefficient for the bacterium, *Pseudomonas extorquens*. For initial methanol concentrations below 10 g l⁻¹, the yield coefficient (Y₅) is inversely proportional to the initial methanol concentration (s₀), so that

\[
Y₅ = 0.25 s₀ + 2.5 \quad \ldots\ldots\ldots\ldots\ldots.(1)
\]

Until recently, insufficient data had been published for methanol-utilizing yeasts, to determine if a similar relationship exists, although yield depression by methanol in both *Candida boidinii* (Volfova and Pilát, 1974; Pilát and Prokop, 1975) and *Pichia pastoris* (Wikén et al., 1977) cultures has been indicated. Pal and Hamdan (1979) have recently reported some studies on the growth of a methanol-utilizing *Hansenula polymorpha* isolated from sea water from the Arabian Gulf. For initial methanol concentrations below 1.0 g l⁻¹ a similar relationship between Y₅ and s₀ can