hizo un *scale down* del proceso fermentativo y se desarrolló un esquema de fermentación semicontinua en el que se redujo el tiempo de fermentación a 40 h, con el mismo nivel de expresión de la proteína. (Figura 2). A partir de este resultado una nueva evaluación de la tecnología para una planta de 2 000 t/año mostró plazos de recuperación de la inversión de 3 años.

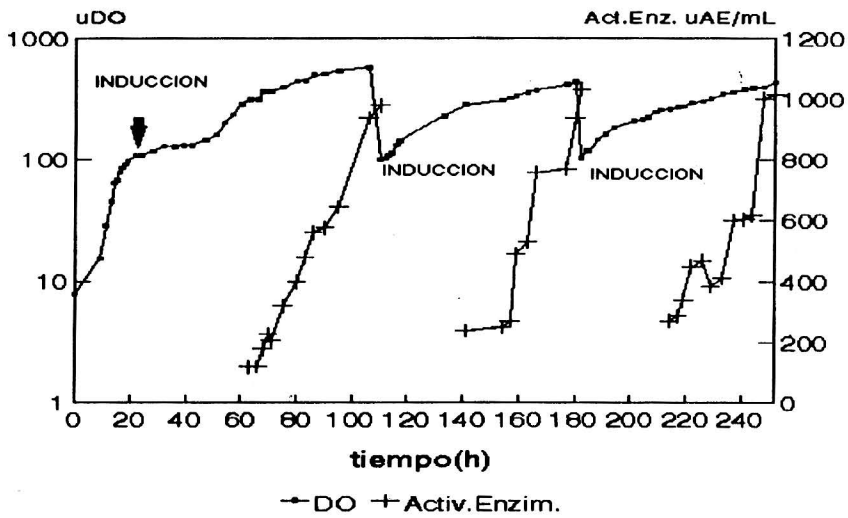

Figura 2. *Proceso de fermentación semicontinuo, en el que disminuyó el tiempo de fermentación a 40 h.*

MOLECULAR GENETICS AND BIOTECHNOLOGY OF METHYLOTROPHIC YEASTS

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In recent years an number of yeast species other than *Saccharomyces cerevisiae* have become accessible for molecular genetics and thereby for potential application in biotechnology. In this respect the methylotrophic yeasts, *Hansenula polymorpha* and *Pichia stipitis* have already been proven to offer significant advantages over *S. cerevisiae* for the production of certain heterologous proteins. The methylotrophic yeasts share general pathways to assimilate and catabolize methanol. Growth on methanol is accompanied by a strong induction of peroxisomes and enzymes involved in methanol metabolism. The strong inducible promoters of the corresponding genes are used for the expression of heterologous genes.

To improve the use of these promoters we have analyzed in great detail the regulation of the MOX promoter of the gene encoding methanol oxidase. We have found methods to circumvent the tight glucose repression of this promoter. In *S. cerevisiae* the MOX promoter can mediate a glucose repressible expression of a fused lac Z gene. This repression was mediated by MOX-B, a 240 bp promoter region which is also involved in catabolite repression in *H. polymorpha*. The negative regulation mediated by MOX-B was counteracted by Adr 1p, a transcription factor which has been shown to be involved in the derepression of ADH2 and, most remarkably, of genes encoding peroxisomal proteins. Details of the binding of Adr 1p to the MOX promoter and its action will be discussed.

During Mox derepression, two different transcripts have been detected starting in the MOX promoter at -25 and -425, from which the smaller transcript accounts for the translation of methanol oxidase. Several small ORFs in the leader sequence of the larger transcript prevent efficient translation. A model for the function of the strong Mox promoter, involving the Adr 1p homologue and a coordinated switch between the two transcription points will be presented.

Finally the suitability of *H. polymorpha* for application in biotechnology will be demonstrated by the discussion of promising developments of pharmaceutical proteins such as the production of hirudin and hepatitis B L- and S-antigenes. Moreover the use of recombinant *H. polymorpha* for bioconversion will be presented.