Biocatalytic preparation of 5-methyluridine (5-MU)

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ABSTRACT
The potential of chemo-enzymatic methods to produce active pharmaceutical ingredients (APIs) such as stavudine (d4T) and zidovudine (AZT) has been demonstrated during this investigation. Compared to conventional chemical synthesis, such methods have the advantage of milder reaction conditions such as low temperature, ambient pressure and pH. However, several challenges need to be overcome, such as low substrate solubility, low enzyme stability and activity under operational conditions, as well as possible substrate and/or product inhibition. This process was successfully scaled up to bench-scale (10 – 20 L), during which the effect of the following physio-chemical variables were evaluated: pH, temperature, substrate concentration and reactor configuration. During the investigation, the effect of increasing reactor productivity to commercially viable levels, albeit at low substrate solubilities, was also demonstrated. The process also demonstrated the isolation of 5-methyluridine (5-MU) from the biocatalytic reaction and integration into the subsequent chemical steps to produce β-thymidine, a key intermediate in the preparation of antiretrovirals. Typically, large costs are associated with the recovery of products from dilute and complex feed streams. The current paper discusses how the above challenges were successfully overcome and implemented at 20 L scale.

OBJECTIVE
To demonstrate the feasibility of carrying out the biocatalytic reaction to produce 5-MU at bench-scale (10 L) while meeting the required reaction performance with respect to guanosine conversion, 5-MU yield, reactor productivity and final product concentration.

INTRODUCTION
Sub-Saharan Africa remains the region worst affected by the HIV/Aids pandemic (see Figure 1), with an estimated 21.6 - 27.4 million people currently living with HIV region. It is estimated that 5.5 million people or 11,9% of the South African population are infected with HIV.

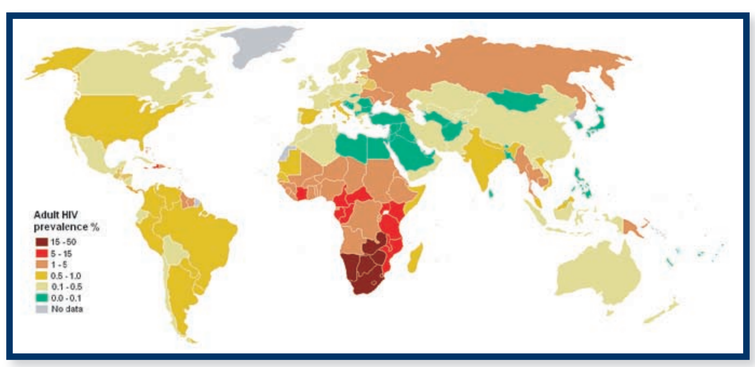
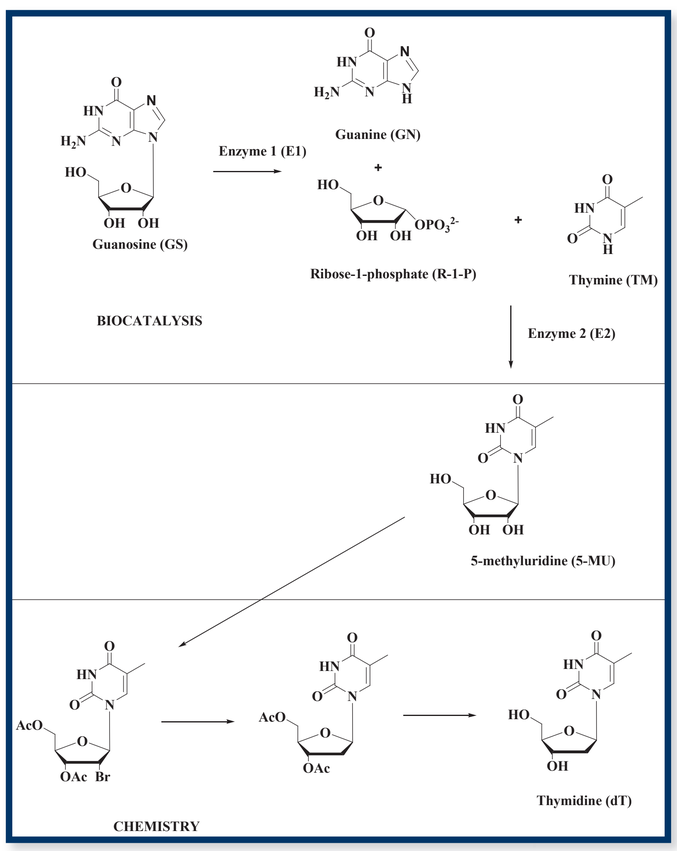


Figure 1: Prevalence of HIV among adults per country at the end of 2005

Stavudine (d4T), zidovudine (AZT), lamivudine (3TC), nevirapine (NVP) and efavirenz (EFV) are widely used in the first line regimen treatment of HIV/Aids. The drugs are generally in combination therapy and represent 96% of the ARV's procured to date, in sub-Saharan Africa[1] to treat HIV/Aids.

β-thymidine is a key intermediate in the preparation of stavudine and AZT. β-thymidine is readily prepared from 5-MU (see Scheme 1). 5-Methyluridine is currently produced by chemical means; however, the methods suffer from several disadvantages such as lengthy process steps; low yields and low selectivities, as a mixture of isomers are produced. An alternative biocatalytic process was thus developed at bench-scale to produce 5-methyluridine (5-MU).



Scheme 1: Chemo-enzymatic preparation of β-thymidine

BIOCATALYSIS
The application of biocatalysis in the preparation of fine chemicals and pharmaceuticals is well documented[2,3]. Biocatalytic processes are increasingly being used to penetrate the chemical industry. A recent study comprising of 134 industrial scale biotransformations carried out a scale >100 kg, using whole cells or enzymes, showed that hydrolases and oxido-reductases dominate industrial applications. An analysis of the average reaction performance based on fine chemicals showed product yields of 78%, final product concentrations of 11% m/m, and volumetric productivities of 15.5 $g.L^{-1}.h^{-1}$.

The biocatalytic process developed to produce 5-MU used a coupled enzyme system E1 and E2 and thus further complicated the development of the process as each enzyme exhibited different thermal and pH activity stability optima. As shown in Figure 2, the low solubility of substrates of between 1,5 -3,0% m/m highlighted the challenge with respect to increasing substrate concentration and reactor productivity to the required level. The possibility of product and/or substrate inhibition of the enzyme also had to be considered as substrate and product concentrations were increased.

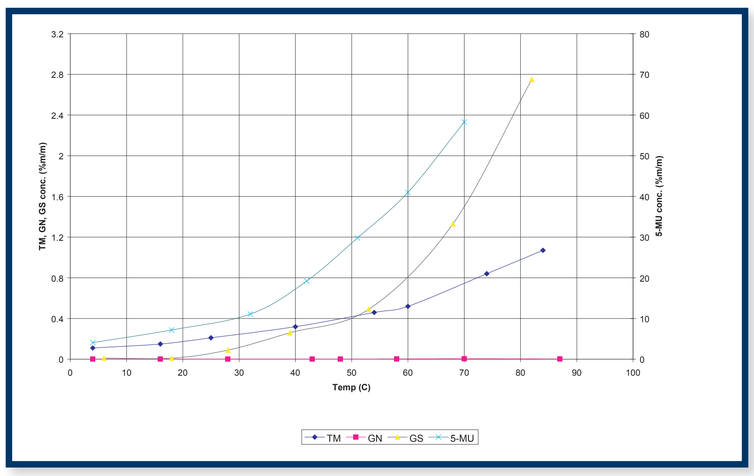


Figure 2: Solubility curve of Thymine (TM), Guanine (GN), Guanosine (GS) and 5-methyluridine (5-MU) in water

RESULTS
The results obtained showed a guanosine conversion of 94.7 ± 2.03 and 5-MU yield of 88,2% ± 6.21. The overall mole balance was 104% ± 7,61%. The biocatalytic reaction was carried out at bench-scale (10 L) and was shown to be robust and reproducible.

Although lower reactor productivities of 7 - 10 $g.L^{-1}.h^{-1}$ were achieved, compared to the process target (see Table 1) the impact was negligible on the process economics.

Process parameter	Process target	Process parameters achieved
Guanosine conversion (%)	90	> 90
Product yield (%)	75	80 - 90
Product concentration (% m/m)	11	7 - 10
Reactor productivity ($g.L^{-1}.h^{-1}$)	15 - 20	7 - 10

Figure 3: Preparation of 5-MU at bench-scale using the CR-16 reactor (10 L)

CONCLUSION
The results obtained demonstrated the successful implementation of a biocatalytic process at bench-scale to prepare 5-methyluridine a key intermediate in the preparation of β-thymidine and ARVs such as stavudine (d4T) and AZT.

CSIR bioscientists have developed an economically-competitive route for the preparation of an intermediate in the production of antiretrovirals.

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