Effects of a nutrient mist bioreactor system on growth kinetics and secondary metabolism of transformed roots of *Artemisia annua*

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Transformed roots are a very promising source of biologically active compounds, but industrial-level production has proved very challenging. *Artemisia annua* produces artemisinin, a sesquiterpene lactone which is a potent antimalarial. Transformed roots of *A. annua* have exhibited increased levels of artemisinin when grown in a nutrient mist bioreactor. Additionally, roots showed no evidence of oxygen deprivation in this gas-phase system. Growth, however, was lower than that observed in oxygen-limited liquid-phase systems. Mathematical characterization of the mist system indicated that the root packing fraction is an important parameter in that sparsely packed roots will not capture enough mist particles to support rapid growth. We have recently shown that roots inoculated at a high packing fraction into a mist reactor grow as well as roots grown in a liquid system. Growth kinetics showed some unusual patterns of metabolism that are not the result of nutrient limitation. The results of these metabolic studies, particularly those relating to carbon uptake, should provide fundamental information for bioprocess engineering and also enhance knowledge in the field of plant biology.

Secondary metabolites and growth

Kim et al. (2001) noted a 3-fold increase in artemisinin accumulation in *A. annua* hairy roots grown in a mist reactor compared to liquid-based systems. While this result alone is worthy of further investigation into the biological responses of roots to mist culture, the main impetus for growing roots in a gas-phase system such as the mist reactor is its ability to eliminate the oxygen deficiency typically found in liquid-phase systems, which limits the ability to achieve a high biomass density. However, roots grown in a mist reactor had lower dry mass compared to those harvested from a bubble column reactor.

Mathematical modeling

Mist deposition on roots, as described by Wyslouzil et al. (1997), is a function of the root diameter and packing fraction; the length of the root bed; the mist particle diameter, density, and diffusivity; and the carrier gas velocity. The volume of mist required by the roots to achieve a desired growth rate depends on the concentration and volumetric feed rate of the limiting nutrient in the medium, the biomass yield on the limiting nutrient, the volume of the growth chamber, and the packing fraction of the roots. The volume of mist captured by the roots must necessarily be equal to or greater than the volume required in order to maintain a given growth rate. Analysis by Kim et al. (2002) suggested that root growth in a mist reactor was being limited by insufficient nutrient availability.

Packing fraction

The nutrient mist bioreactor described by Weathers et al. (1999) was modified such that the growth chamber was replaced with a much smaller cylinder into which roots were manually inoculated at an initial packing fraction of 0.29. Kim et al. (2002) used a hybrid liquid- and gas-phase reactor which was first run as a bubble column to allow roots to become immobilized before switching to gas-phase operation which commenced at packing fractions of no higher than 0.05. The average specific growth rate in this system was 0.07 day^{-1}, while in the smaller modified mist reactor it was 0.12 day^{-1}. While direct comparison between the two systems is difficult due to disparity in culture times and other operating conditions, the results suggest that initial inoculum density influences subsequent growth in mist reactors.

Limiting nutrient concentration

The volume of mist required by the roots may be reduced by increasing the concentration of the limiting nutrient in the medium. It was assumed that the major limiting nutrient was the carbon source, sucrose. When roots were grown in the modified growth chamber, average specific growth rates after 6 days were 0.12 day^{-1} and 0.18 day^{-1} for media supplemented with 3% or 5% sucrose, respectively (Figure 1). Studies are currently underway to determine whether increasing the sucrose concentration further will increase the growth rate, or at least allow the roots maintain the apparent growth rate “peak” achieved by day 4. Note the subsequent decrease even though media analysis has shown that no major nutrients have been exhausted. Shake flask studies indicated that maximum growth is attained with 6% sucrose; higher concentrations were inhibitory. However, we postulate that the roots may tolerate higher concentrations in the mist reactor since the method of nutrient delivery is dramatically different. In a mist system, roots are not immersed in liquid and detrimental osmotic effects may be lessened.

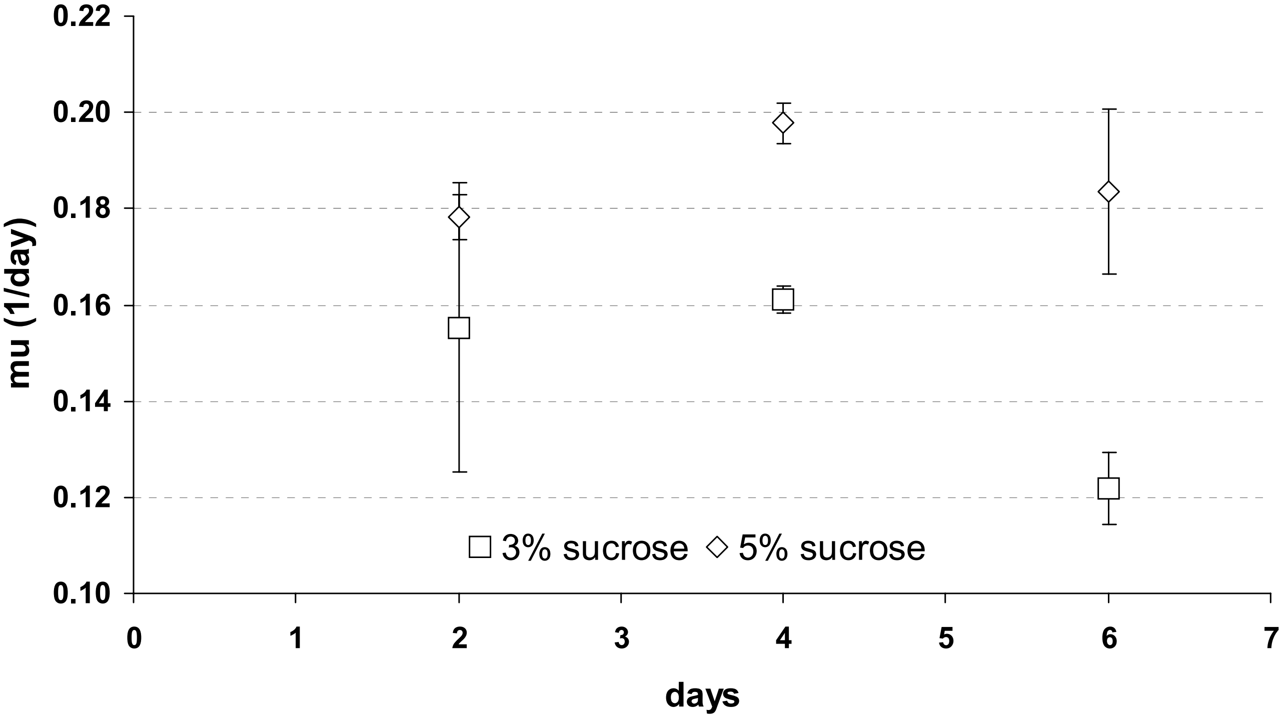

Figure 1. Specific growth rates (ln [final dry weight / initial dry weight] / time) of *A. annua* roots grown in the modified mist reactor with 3% or 5% sucrose.

Sugar consumption

After residual sugar content from the medium of roots grown in shake flasks with various initial sucrose concentrations was analyzed, an interesting trend was noted. As long as there was still sucrose present in the medium, the ratio of the component monosaccharides, glucose and fructose, was approximately 0.85 (Figure 2). Examination of residual sugar data from bubble column and mist reactors (Kim, 2001) and the modified growth chamber showed the same trend. However, shake flask studies of roots grown exclusively on each individual sugar showed reduced growth on glucose versus fructose (Weathers et al., 2004), which leads one to question why many roots, including *A. annua*, preferentially consume glucose before fructose when both are present. Shake flask studies where roots are provided equivalent sucrose concentrations of only glucose and fructose at a ratio of 0.85 are currently in progress. It may be advantageous to provide sugars to the roots in this manner rather than require the roots to expend energy and resources in order to achieve this ratio.

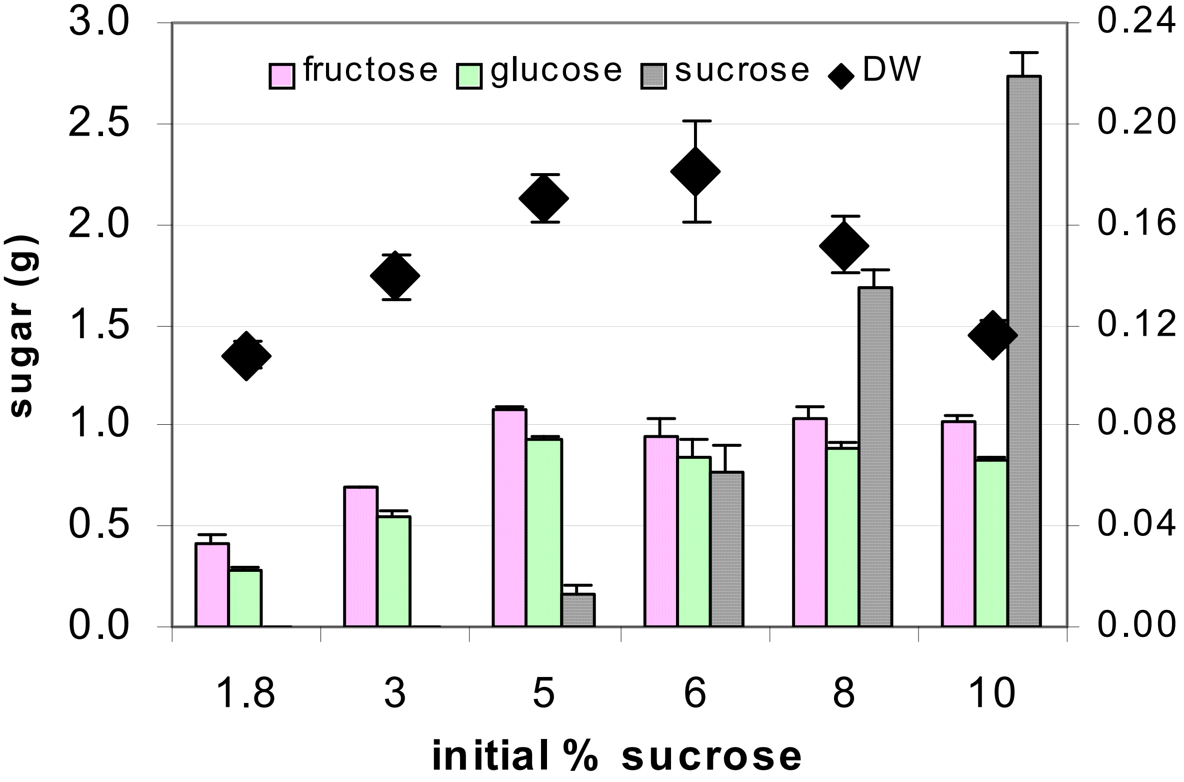

Figure 2. Residual sugar profiles and final dry weights of *A. annua* roots grown for 14 days in shake flasks containing varying concentrations of sucrose.

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