Effects of inoculum density, carbon concentration, and feeding scheme on the growth of transformed roots of *Artemisia annua* in a modified nutrient mist bioreactor

by

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ABSTRACT

Previous work has shown that despite the lack of oxygen limitation, transformed roots of *Artemisia annua* had lower biomass productivity in a nutrient mist bioreactor than in a liquid-phase bubble column reactor where the roots demonstrated metabolic signs of oxygen stress. Mathematical modeling suggested that the roots were too sparsely packed to capture mist particles efficiently and to achieve high growth rates. In this study, higher packing fractions were tested, and the growth rate increased significantly. Similarly, higher sucrose concentrations increased the growth rate. Growth kinetics for 2, 4, and 6 days showed an unexpected decrease or stationary growth rate after only 4 days for both 3% and 5% sucrose feeds. Residual media analyses indicated that carbon was not exhausted, nor were other major nutrients including phosphate. Increasing the misting frequency such that the total amount of carbon delivered from a 3% sucrose feed was equivalent to that delivered in a 5% sucrose feed showed that growth was affected by the modified cycle. These studies showed that both the concentration of carbon source and alteration of misting frequency can significantly increase growth rates of hairy roots in mist reactors.

ACKNOWLEDGEMENTS

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(p.s. *quack*). Love to my wonderful Foldies who kept me company during the never-ending string of all-nighters, to those cats who have crossed the Rainbow Bridge, and to people in the cat fancy who encouraged me, especially Nancie. Special love and thanks to my family for supporting me financially and emotionally, and not pushing me to do anything I didn't want to do. You don't know how much that meant to me. True caring is about putting another person's needs and well-being above all. I dedicate this to my grandmother, who did not live to see this day, but did get to see me dance for her. She would have been proud no matter what I did, and that is one of the things I loved most about her.

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In closing, a quote from Eeyore: "Sitting on thistles doesn't do them any Good. Takes all the Life out of them." Interpret that any way you see fit. Few truly appreciate Eeyore's profundities.

"Thanks for noticin' me."

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Introduction and literature review

(the major portion of this chapter is taken from an invited review coauthored by Towler, M.J., Kim, Y.J., Correll, M.J., Wyslouzil, B.E. and Weathers, P.J. and published as: Design, development, and applications of mist bioreactors. In: Plant tissue culture engineering (ed. S.D. Gupta), Kluwer Academic Publishers, The Netherlands. (2005))

Aeroponic technology has been used extensively to study biological phenomena in plants including drought stress, symbiotic relationships, mycorrhizal associations, disease effects, mineral nutrition, overall plant morphology and physiology (Weathers and Zobel, 1992), and some work has also been completed with animal tissue culture (Friberg et al., 1991). Aeroponics offers many advantages to whole plant growth because of the enhanced gas exchange that is provided. The focus here is on the use of aeroponics (nutrient mists) for *in vitro* culture of differentiated tissues; specifically, the culture of transformed (hairy) roots.

There are two main categories of bioreactors: liquid-phase and gas-phase reactors (Kim et al., 2002b). In liquid-phase reactors, the tissue is immersed in the medium. Therefore, one of the biggest challenges in a liquid-phase culture is delivering oxygen to the submerged tissues due to low gas solubility. In gas-phase reactors (which include nutrient mist culture), the biomass is exposed to air or a gas mixture and nutrients are delivered as droplets. Droplet sizes can range from 0.01-10 μm for mists, 1-100 μm for fogs, and 10-10^3 μm for sprays (Perry and Green, 1997). The mass transfer limitation, especially of oxygen, can be significantly reduced or eliminated by using a gas-phase culture system (Weathers et al., 1999).

Mist reactor configurations

The original design of aeroponics systems dispersed nutrient medium via spray nozzles that required compressed gas and were prone to clogging by medium salts (Weathers and Zobel, 1992), while later mist reactors used submerged ultrasonic transducers. In the early mist reactors (Figures 1.1A & 1.1B), the ultrasonic transducer was in direct contact with nutrient medium salts and had to be autoclaved, considerably shortening the life of the transducer (Tisserat et al., 1993; Woo and Park, 1993; Buer et al., 1996). Buer et al. (1996) fabricated an acoustically transparent polyurethane window to isolate the medium from the transducer (Figure 1.1C) but making the windows was difficult, time consuming, and the starting materials were expensive. Chatterjee et al. (1997) replaced the custom window with an inexpensive, commercially available polypropylene container (Figure 1.2) and this design was successfully used for both hairy root (Chatterjee et al., 1997) and micropropagation studies (Correll et al., 2001; Correll and Weathers 2001a; 2001b). Similarly, Bais et al. (2002) used a polycarbonate GA-7 vessel. The nutrient mist system currently used by Weathers et al. (1999) (Figure 1.3) has an acoustic window consisting of a thin teflon sheet that has a higher temperature tolerance than polypropylene and can also be incorporated into a reactor of almost any size or shape. The designs of the mist reactor configuration have evolved as the applications of these systems have become more varied.

Mist reactors for hairy root culture

A number of valuable pharmaceuticals, flavors, dyes, oils, and resins are plant-derived secondary metabolites. Since secondary metabolites are usually produced by specialized cells and/or at distinct developmental stages (Balandrin et al., 1985), plant

cell suspension cultures are not usually practical sources of these chemicals. Hairy root cultures can have the same or greater biosynthetic capacity for secondary metabolite production compared to their mother plants (Banerjee et al., 1998; Kittipongpatana et al., 1998). Indeed, hairy roots have been considered potential production sources for important secondary metabolites (Flores and Curtis, 1992). A summary of studies using hairy roots in mist reactors is provided in Table 1.1. In nearly all cases, hairy root growth in mist reactors was as good as or better than liquid-phase cultures.

Secondary metabolism of hairy roots grown in various bioreactors has been recently reviewed by Kim et al. (2002b). Kim et al. (2001) noted a 3-fold increase in artemisinin accumulation in mist reactors, and subsequently, Souret et al. (2003) provided a further analysis when they compared the expression levels of four key terpenoid biosynthetic genes in *A. annua* hairy roots grown in mist reactors versus liquid-phase systems. Although there was notable heterogeneity in terpenoid gene expression, the differences could not be attributed directly to one single factor and were likely the result of complex interactions of multiple factors including oxygen status, presence or absence of light, culture age, and tissue location within the growth chamber of the bioreactor. Bais et al. (2002) and Palazon et al. (2003) likewise noted alterations in secondary metabolite content when hairy roots of *Cichorium* and *Panax*, respectively, were grown in mist reactors.

Several hairy root lines can develop mature chloroplasts capable of photosynthesis (Flores et al., 1993), and these green roots have different metabolic capabilities compared to non-green roots, although response to light is not necessarily dependent on whether the roots turn visibly green. In addition, light can have a significant effect on growth of hairy

roots (Taya et al., 1994) and many enzymes in the biosynthetic pathways for secondary metabolites are regulated by light (Kim et al., 2002b). However, delivery of light into a bioreactor, especially one that is densely packed with roots, is problematic. Interestingly, the roots themselves may have light-guiding properties (Mandoli and Briggs, 1982; 1983). *A. annua* hairy roots were able to transmit light from a helium-neon laser through the interior of the root (Weathers and Swartzlander, unpublished), indicating that roots may have the ability to function as leaky optical fibers.

The morphological characteristics of hairy roots demand special consideration with regards to bioreactor design. The mist reactor provides a low-shear environment for growing hairy roots and reduces gas-exchange limitations normally found in liquid-phase bioreactors. Studies by McKelvey et al. (1993) suggested that roots are more capable of compensating for poor liquid dispersion than for poor gas dispersion within reactor systems (Curtis, 1993). An economically viable production scheme depends in part on the ability to attain a high biomass density. The maximum root tissue concentration that can be achieved is dependent on the delivery of oxygen and other nutrients into the dense matrix (Curtis, 2000). Gas-phase reactors such as the mist reactor can virtually eliminate any oxygen deficiency in dense root beds (Weathers et al., 1999). Kim et al. (2002a), however, noted that the availability of non-gaseous nutrients may be a concern; i.e. gas dispersion is improved at the expense of liquid dispersion. Furthermore, it is difficult to uniformly distribute roots in the growth chamber of a gas-phase reactor without manual loading (Kim et al., 2002b). Several groups (Flores and Curtis, 1992; Ramakrishnan et al., 1994; Wilson, 1997; Kim et al., 2002a; Ramakrishnan and Curtis, 2004) circumvented this issue with hybrid liquid- and gas-phase reactors which were first

operated as liquid-phase systems to allow the roots to circulate, distribute, and/or attach to immobilization points. Gas-phase operation could then be initiated as desired, usually when the liquid-phase reactor was no longer effective at supporting root growth due to limitations in nutrient delivery to the dense root beds. Towler and Weathers (2003; see Chapter 2) have also described a method by which roots may be quickly attached to a mesh support, thereby allowing mist mode to commence shortly after inoculation.

The gas phase surrounding tissues also plays a key role in the culture and secondary metabolite productivity of hairy roots (see review by Kim et al., 2002b). One of the major advantages of the mist reactor is the ability to alter the gas composition. Oxygen is essential for respiration and thus, the growth of roots. To assess the response of hairy roots to altered levels of oxygen in mist reactors, alcohol dehydrogenase (ADH) mRNA, an indicator of oxygen stress, was measured in *A. annua* hairy roots. Comparison of ADH mRNA expression in both shake flasks and bubble column reactors to mist reactors indicated that the mist-grown roots were not oxygen limited (Weathers et al., 1999). Roots grown in the mist reactor to a density of about 37% (v/v) had no detectable expression of ADH (Kim, 2001), whereas ADH mRNA was detected in roots from the bubble column at packing densities as low as 6% v/v (Weathers et al., 1999). Roots grown in the bubble column reactor, however, had higher dry mass compared to those harvested from the mist reactor. This unexpected result may be explained through modeling of mist deposition dynamics (see Mist deposition modeling, below).

In addition to oxygen, carbon dioxide also affects the growth of hairy roots. CO_2-enriched nutrient mist cultures of *Carthamus tinctorius* and *Beta vulgaris* hairy roots showed increased growth versus control cultures that were fed ambient air (DiIorio et al.,

1992a). However, a similar effect was not observed in hairy roots of *Artemisia annua*. When grown in mist enriched with 1% CO_2, root growth was not significantly different than that of roots grown in ambient air (Wyslouzil et al., 2000), although visually the roots appeared much healthier and there was a change in the branching rate. Kim et al. (2002a) also noted similar results where the biomass accumulation was similar between root cultures grown in ambient air and those supplemented with 0.5% CO_2. It is possible that perhaps the optimum level of CO_2 enrichment for *A. annua* hairy roots was not provided to these cultures, particularly considering that the response of roots to CO_2 can vary depending on species and growth environment (DiIorio et al., 1992a; Weathers and Zobel, 1992).

Ethylene accumulation may also be involved in regulating biomass and secondary metabolite production. Although all plant tissues can both produce and absorb the gaseous phytohormone ethylene, which has profound effects on growth, development, and even the production of secondary metabolites (Weathers and Wyslouzil, 2000), some species of plants may produce more ethylene than others. Indeed, Biondi et al. (1997) showed that hairy roots of *Hyoscyamus muticus* produced 3 times more ethylene than untransformed roots, and growth of *A. annua* hairy roots was significantly reduced by ethylene (Weathers et al., 2005). Sung and Huang (2000) showed that hairy roots of *Stizolobium hassjoo* had lower biomass and produced lower levels of secondary metabolites when ethylene was allowed to accumulate in the headspace of the culture vessel. Recently, we also observed that ethylene, provided as ethephon, significantly inhibited both growth and artemisinin production in *A. annua* hairy roots (Weathers et al., 2005). Considering that ethylene production is inhibited by CO_2, it is possible that the

stimulation in root growth by higher levels of CO_2 is the result of inhibition of ethylene biosynthesis. Designs in reactors that scrub ethylene from the gas phase may further improve hairy root growth and promote secondary metabolite production.

Mist deposition modeling

Droplet transport and deposition in a bed of hairy roots may limit growth if an adequate supply of nutrients does not reach the surface of all roots. Consequently, mist deposition is a key step in the mass transfer of nutrients to the roots in a mist reactor (Wyslouzil et al., 1997). The standard aerosol deposition model for fibrous filters was applied to mist deposition in hairy root beds by Wyslouzil et al. (1997). The ideal filter has evenly distributed fibers that lie perpendicular to the flow. Though root beds have regions of high and low packing density and grow in all directions, the model can still be used to study the qualitative trends of mist deposition behavior. When the model was tested on root beds that had been manually packed to $\alpha = 0.5$ (α = volume fraction occupied by roots), it was found to correspond well to experimental data as long as the Reynolds number (Re), based on the root diameter, was < 10. The Reynolds number characterizes the relative importance of inertial and viscous forces, and for filtration problems:

$$\mathrm{Re} = \frac{\rho U_o D_R}{\mu_g} \qquad [1.1]$$

where ρ and μ_g are the density and viscosity of the carrier gas, D_R is the diameter of the root, and U_o is the gas velocity in the root bed. In terms of the number of droplets captured, the efficiency (η_B) of the root bed is a function of the particle diameter (D_P) and is equal to:

$$\eta_B = 1 - \exp\left[\frac{-4L\alpha\eta_C}{D_R(1-\alpha)}\right] \qquad [1.2]$$

where L is the length of the root bed, and:

$$\eta_C = 1 - (1-\eta_{IMP+INT}) \times (1-\eta_D), \qquad [1.3]$$

the combined capture efficiency due to impaction, interception, and diffusion, respectively. Determining $\eta_{IMP+INT}$ involves solving two nonlinear equations as found in Crawford (1976), and the expression for calculating η_D is given in Friedlander (1977). The overall mass deposition efficiency (η_{OM}) of the root bed is the product of the root bed efficiency $\eta_B(D_{Pi})$ and the mass fraction $m(D_{Pi})$ of mist particles of diameter D_{Pi} summed over the aerosol size distribution data:

$$\eta_{OM} = \sum_i \eta_B(D_{Pi}) \times m(D_{Pi}). \qquad [1.4]$$

Typical mist particle size data were obtained experimentally by Wyslouzil et al. (1997). The amount of medium captured by the roots (V_{dep}) in mL per day is:

$$V_{dep} = 24\omega \times Q_L \times \eta_{OM} \qquad [1.5]$$

where 24 is the conversion factor from hours to days, ω is the duty cycle in minutes per hour, and Q_L is the medium flow rate in mL per minute while misting is occurring. The amount of medium required to support the growth of roots (V_{req}) depends on: the density of the roots ρ_{FW} (grams fresh weight per mL), the dry weight / fresh weight ratio (DW/FW), the specific growth rate μ (day^{-1}), the nutrient concentration in the medium C_s (g per L), the apparent biomass yield of the growth-limiting nutrient $Y_{X/S}$ (g DW biomass per g nutrient consumed), the working volume of the reactor V (L), and packing fraction α. The expression for V_{req} is:

$$V_{req} = 10^6 \rho_{FW} \times \frac{DW}{FW} \times \frac{\mu}{C_S} \times \frac{1}{Y_{X/S}} \times V \times \alpha . \qquad [1.6]$$

The growth-limiting nutrient is assumed to be sugar. Clearly, V_{dep} must be equal to or greater than V_{req} in order to maintain a desired growth rate μ.

Kim et al. (2002a) applied the model to *A. annua* hairy roots grown in the nutrient mist bioreactor, and it suggested that growth was limited by insufficient nutrient availability. This hypothesis has been tested in several ways (Towler, this thesis). Since V_{dep} is a function of the packing fraction (α), increasing α should increase V_{dep} and thus support a higher growth rate by allowing more nutrients to be captured by the roots. To test this hypothesis, the nutrient mist bioreactor described by Weathers et al. (1999) was modified whereby the growth chamber was replaced with a much smaller (~45 mL volume, ~30 mm diameter) cylinder into which roots were manually inoculated at an initial packing fraction of 0.29. The system was then immediately run in mist mode rather than as a hybrid liquid- and gas-phase reactor. Those studies are described here (Chapter 3). While Kim et al. (2002a) commenced mist mode at packing fractions that were at most 0.05 and observed an average specific growth rate of 0.07 day^{-1}, the average growth rate in the modified mist reactor was 0.12 day^{-1} for a 6-day period (see Chapter 3). Due to the disparity in culture times and other operating conditions, direct comparison between these systems is difficult; however, roots grown in the modified mist reactor had higher growth rates compared to those obtained by Kim et al. (2002a), thereby supporting the hypothesis that initial inoculum density influences subsequent growth in mist reactors.

Alternatively, since V_{req} is inversely proportional to the concentration of the limiting nutrient C_S, increasing C_S should decrease V_{req}. Using the smaller modified mist reactor previously described, *A. annua* hairy roots were fed medium containing either 3% or 5% sucrose. After 6 days, roots grown with 5% sucrose had a significantly higher specific growth rate compared to roots grown in 3% sucrose (0.18 $days^{-1}$ and 0.12 $days^{-1}$ for 5% and 3% sucrose, respectively). The growth rate dependence on sucrose concentration is examined further in Chapter 3.

While the model suggests that lengthening the duration of the misting cycle increases the amount of nutrients delivered to the roots and should thereby increase growth, this solution is actually more complex. For reasons as yet unknown, the misting cycle plays a significant role in the successful operation of a mist bioreactor. Liu et al. (1999) found that a misting cycle of 3 min on / 30 min off was the optimum of those tested for transformed roots of *A. annua* grown in their nutrient mist bioreactor, though its design and operating conditions were different than those implemented by Weathers et al. (1999). Liu et al. (1999) provided gas either only when mist was not being generated, or continuously; while Weathers et al. (1999) provided gas only when the mist was provided. Interestingly, DiIorio et al. (1992b) also observed that hairy roots seemed to have optimum mist duration for growth. Their studies with hairy roots of *Beta vulgaris* and *Carthamus tinctorius* showed that either increasing or decreasing the "off" time beyond a certain limit adversely affected root growth of those species. Chatterjee et al. (1997) found that a mist cycle of 1 min on / 15 min off caused transformed roots of *A. annua* to darken and become necrotic after 12 d. Yet, studies with a single transformed root of *A. annua* (Wyslouzil et al., 2000) showed that a mist cycle of 1 min on / 15 min

off promoted healthier-looking roots and higher fresh final biomass yields versus the other cycles tested. Studies described here (see Chapter 3) in which the misting cycle was modified so that the mass flow rate of sucrose was maintained while the sucrose concentration varied indicated that root growth could be increased by increasing the length of misting cycle while decreasing the mist off time. These results support the hypothesis that in a mist reactor, higher growth yields can be achieved with increased droplet deposition and by manipulating the on/off cycle period.

Droplet size and orientation of flow must also be considered for optimal growth and secondary metabolite production of hairy root cultures. If the droplet size is too large, the formation of a liquid layer along the root surface will impede gas transfer to the roots and the system will behave as if it were a liquid-phase reactor (Weathers and Wyslouzil, 2000). Similarly, when mist is provided in an upward direction, the mist can coalescence on the roots closest to the mist feed with less mist reaching the tissue in the higher layers of the growth chamber. Liu et al. (1999) constructed an upward-fed mist reactor with three layers of stainless steel mesh to support the roots, and found that there was a greater than 50% decrease in biomass between the first (bottom) layer and the second and third layers. It is also likely that as the root bed becomes very dense, the lower sections will accumulate liquid and essentially become submerged. Mist reactors that are top fed have the advantage of co-current down-flow of gas and liquid phases along with gravity which facilitates drainage. In contrast, top versus bottom mist feeding seems to be of less consequence in micropropagation systems and the orientation chosen is often a matter of convenience.

Another factor that may play a role in the growth of hairy roots is that of conditioned medium. Both Chatterjee et al. (1997) and Wyslouzil et al. (2000) used autoclaved medium with varying degrees of pre-conditioning by pre-growing roots in the medium before using it in subsequent experiments. Wyslouzil et al. (2000) also showed that there were higher branching rates when roots were grown in conditioned medium versus fresh medium. The identity of these "conditioning factors" remains elusive, although studies have characterized some of them as oligosaccharides (Schroder et al., 1989), peptides (Matsubayashi and Sakagami, 1996), and auxins (Weathers et al., 2005). For consistency, it is recommended that fresh, filter-sterilized medium be used in all experiments (Weathers et al., 2004). Work from our lab has routinely used filter-sterilized medium for experiments since 1999.

Conclusions

Plant tissues are highly responsive to gases in their environment, especially O_2, CO_2, and ethylene. Due to the low solubility of these gases, mass transfer of these gases to the roots is hindered in a liquid system. Attempting to enhance gas transport by stirring, bubbling, or sparging the liquid can damage shear-sensitive plant tissues. Therefore, gas-phase reactors show many advantages over liquid-phase reactors, especially in terms of the ability to easily manipulate gas composition and allow effective gas exchange in densely growing biomass. However, the interactions between plant tissues and the nutrient mist environment can be complex with many differing design aspects dictated by the application. For example, compare the design of the growth chamber and the misting regimens required for growing hairy roots vs. micropropagated

plantlets (Figures 1 and 2). A better understanding of the biological responses of the cultured tissues must be developed in order for mist reactors to be exploited to their fullest potential. Recent results are promising and further studies are warranted.

Objectives and organization of thesis

The objectives of the work presented in the subsequent chapters are as follows. For the purposes of studying the growth of transformed roots in a gas-phase reactor, it was desirable to decrease or eliminate the liquid-phase immobilization and growth period prior to the switch to aeroponic mode. This was accomplished by devising a method in which the roots attach to a growth matrix very quickly, thereby allowing the removal of the liquid phase and initiating gas-phase culture almost immediately. This technique is described in Chapter 2. It became apparent, however, that the initial root biomass density in the mist reactor was a key parameter to study with regards to its effects on nutrient capture and subsequent growth, so rapid immobilization was not pursued further. Instead, the size of the growth chamber was reduced and it was manually packed with roots at various initial densities. Based on mathematical modeling of mist deposition on roots, several other parameters were also deemed worthy of investigation, including the concentration of nutrients in the growth medium and the mist delivery rate. The responses of the roots to the experimental conditions were evaluated by not only monitoring biomass accumulation, but nutrient consumption as well. This allowed the determination of growth efficiency, or the ability of the roots to use the nutrients provided to their highest potential. The rationale here was to bear in mind that if high biomass production cannot be attained in a cost-effective manner, use of the growth

system will not be commercially feasible. These analyses are discussed in Chapter 3. Lastly, since research undertaken to answer one question always seems to beget even more questions, Chapter 4 provides some thoughts on future work and recommendations for further research.

Table 1.1. Summary of hairy root mist reactor studies

Species	System	Main Results	Reference
Artemisia	acoustic window[1] mist reactor	growth comparable to flasks and plates	Buer et al., 1996
Artemisia	submerged ultrasonics	modified inner-loop reactor growth comparable to flasks	Liu et al., 1999
Artemisia	acoustic window[2] mist reactor	no O_2 limitation, but 50% less biomass than liquid systems	Weathers et al., 1999
Artemisia	acoustic window[2] mist reactor	altered branching rate versus flasks	Wyslouzil et al., 2000
Artemisia	acoustic window[2] mist reactor	3x higher artemisinin content than bubble column	Kim et al., 2001
Artemisia	acoustic window[2] mist reactor	growth comparable to bubble column	Kim et al., 2002a
Artemisia	acoustic window[2] mist reactor	altered terpenoid gene expression versus flasks	Souret et al., 2003
Beta	submerged ultrasonics	growth comparable to flasks	Weathers et al., 1989
Carthamus	submerged ultrasonics	growth comparable to flasks; 15% faster than airlift reactor	DiIorio et al., 1992b; Weathers et al., 1989
Cichorium	acoustic window[3] mist reactor	higher biomass and esculin content than bubble column	Bais et al., 2002
Datura	droplet reactor	1.6x lower doubling time than submerged cultures	Wilson et al., 1990
Datura	hybrid submerged/droplet reactor	successful large-scale (500 L) culture	Wilson, 1997
Fragaria	mist reactor	biomass yield higher than droplet bioreactor	Nuutila et al., 1997
Hyoscyamus	hybrid trickle bed reactor	753 g FW L^{-1} achieved in fed-batch / O_2-enriched system	Ramakrishnan and Curtis, 2004
Hyoscyamus	spray reactor	growth comparable to shake flasks	McKelvey et al., 1993
Nicotiana	spray reactor	50% lower doubling time than flasks	Whitney, 1990, 1992
Panax	spray reactor	altered ginsenoside pattern versus native rhizome	Palazon et al., 2003

1, Conap's EN6; 2, Teflon; 3, polycarbonate

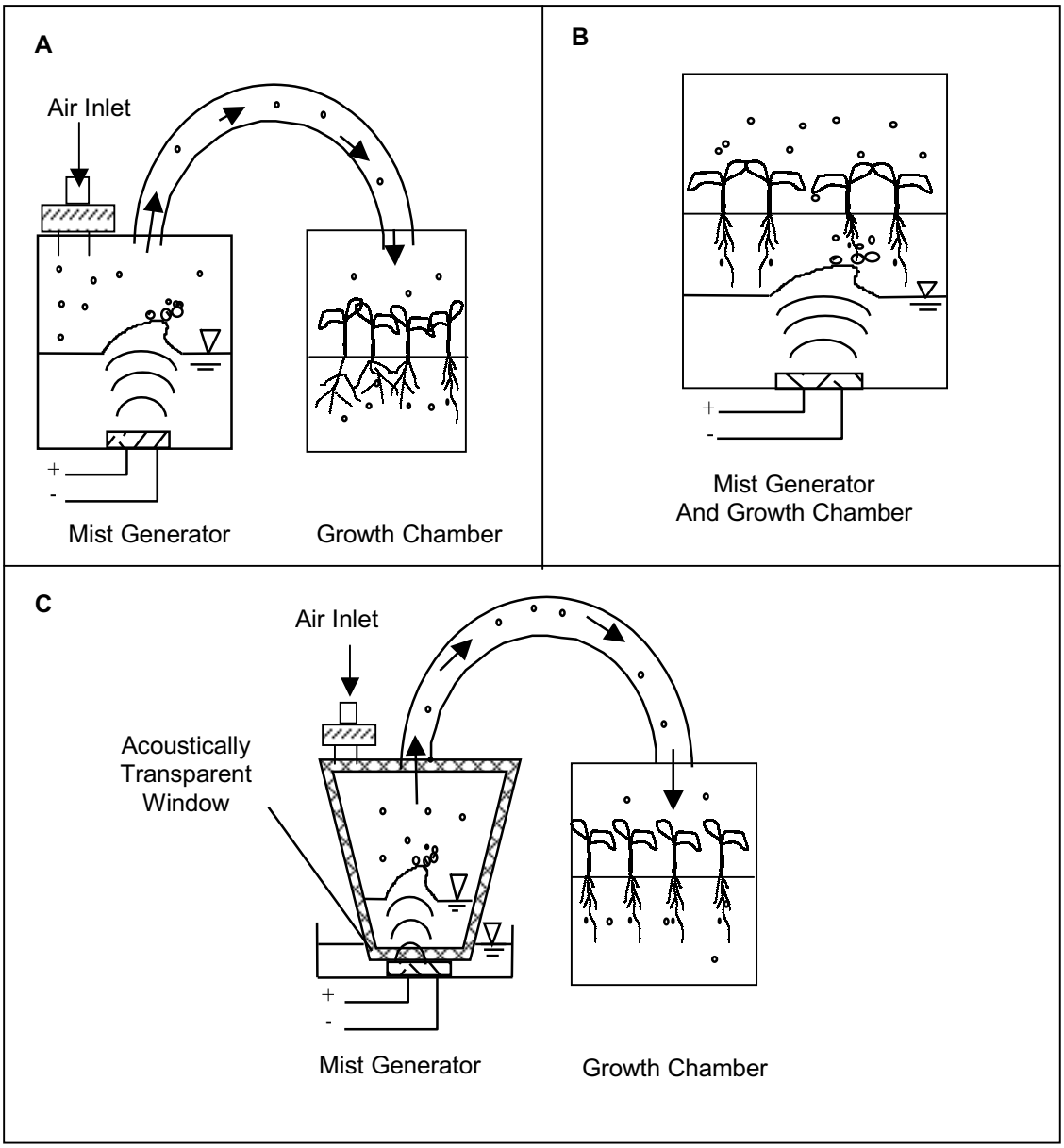

Figure 1.1. Three types of ultrasonic mist reactors: the mist generator and the growth chamber are in separate vessels (A); mist generator and growth chamber are in the same vessel (B); and the transducer is separated from autoclaved components by an acoustic window (C). Direction of mist movement is indicated by arrows. Figure courtesy of M. Correll.

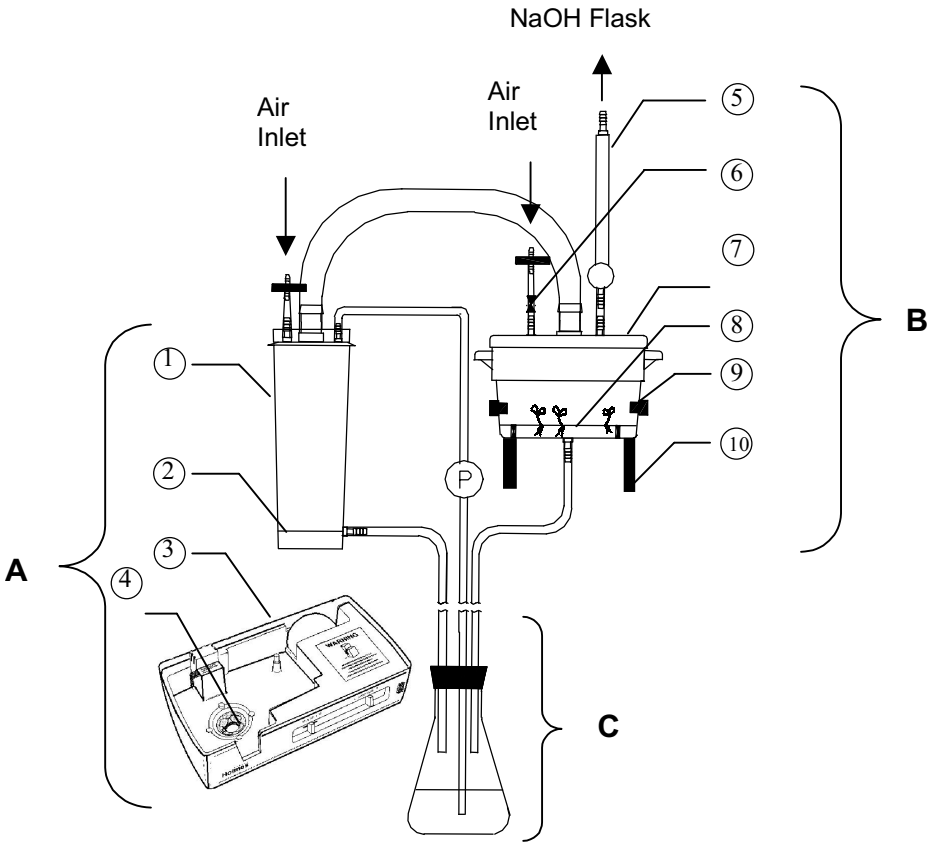

Figure 1.2. Acoustic window mist reactor. A, mist generator; B, micropropagation chamber; C, media reservoir; 1, polypropylene mist chamber; 2, nutrient medium level; 3, Holmes® humidifier base; 4, ultrasonic transducer; 5, coalescer; 6, one-way valve; 7, micropropagation chamber; 8, plant platform; 9, gas sampling port; 10, chamber supports; P, peristaltic pump used for pumping medium to mist chamber. Figure courtesy of M. Correll.

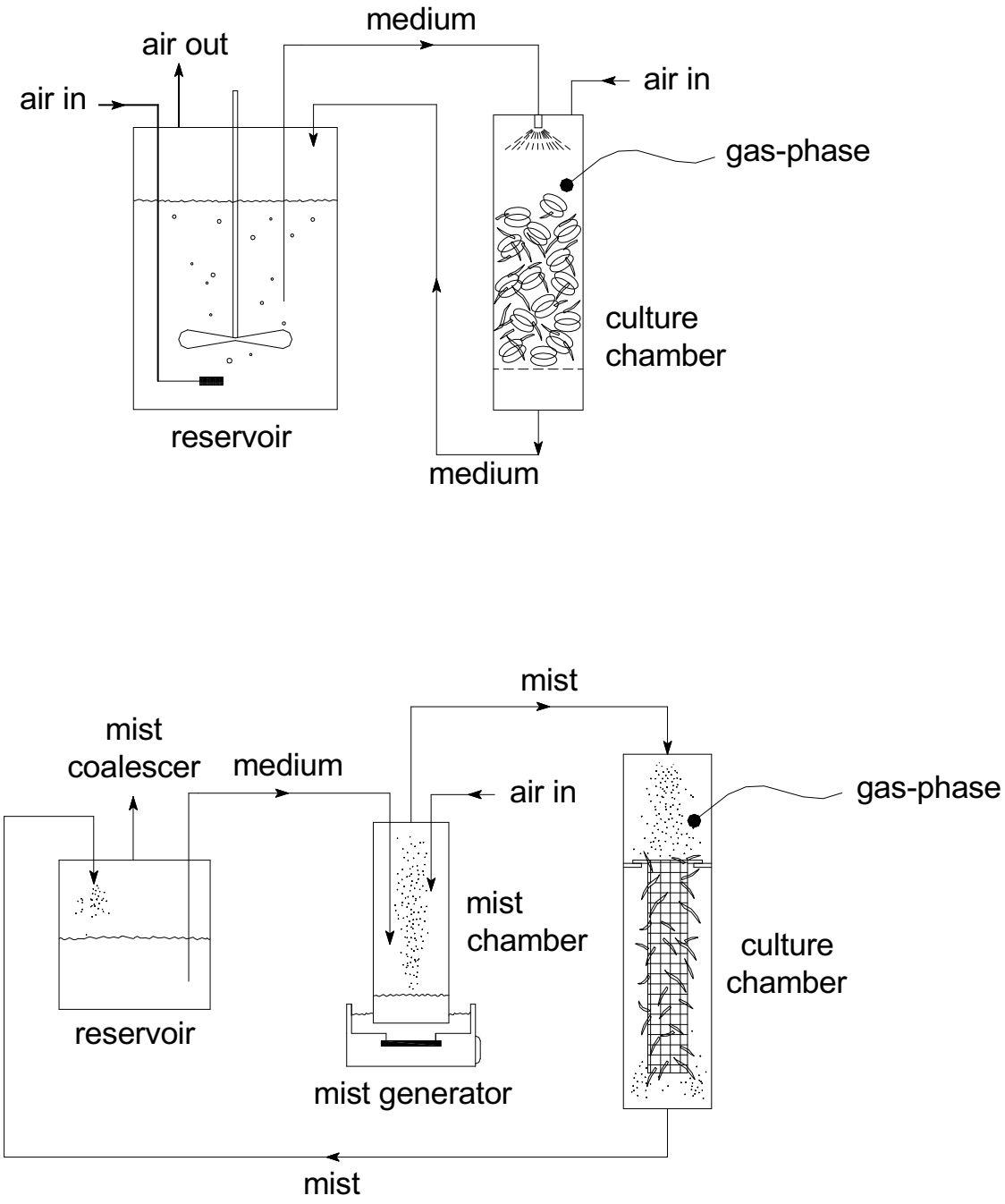

Figure 1.3. Two types of gas-phase bioreactors for hairy root culture. Top, trickle bed reactor. Bottom, nutrient mist reactor. Figure courtesy of Y. Kim.

2

Adhesion of plant roots to poly-L-lysine coated polypropylene substrates

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Abstract

The ability to immobilize plant tissue in a bioreactor is an important process tool. We have shown that roots of several species rapidly attach to poly-L-lysine coated polypropylene mesh in a liquid environment. Using transformed roots of *Artemisia annua* as a model, the attachment process was found to be enhanced by sheep serum but not BSA, and inhibited by excess Mn^{2+} but unaffected by Ca^{2+} or Mg^{2+}. Attempts to characterize the molecule(s) responsible for binding using lectins and antibodies showed that the binding site does not appear to be glycosylated or vitronectin-like. This method of rapid attachment should prove useful for controlled immobilization of roots in bioreactors.

Introduction

Many biologically active compounds accumulated in plants have been used as phytopharmaceuticals (Pereira *et al.,* 2000). Hairy roots have been suggested as a desirable source of these chemicals because they can produce compounds normally synthesized in roots as well as in other parts of the whole plant. Additionally, higher levels of secondary metabolites can be obtained in differentiated tissue cultures as compared to undifferentiated cell suspension cultures (Shanks and Morgan, 1999).

Though many reactors have been used to grow hairy roots, they can be divided into three major types (see review by Kim *et al.*, 2002b). Liquid-phase types include: stirred tanks, air-sparged vessels, and rotating drums. Gas-phase (or liquid-dispersed) types include: nutrient mist, droplet, trickle-bed or trickling film, and drip-tube reactors. The third type includes hybrid reactors, where gas and liquid phases are used at different points during the culture period. Whenever roots are grown in liquid-phase reactors, they remain suspended and can become evenly distributed, ultimately filling the growth chamber and effectively becoming immobilized. Gas-phase reactors, however, pose a challenge; if there is no supporting liquid phase after inoculation, the roots settle to the bottom of the reactor, resulting in uneven biomass distribution that can lead to channeling and limitations in mass transfer. Certainly immobilization of the root inoculum on a support would facilitate a more even distribution of the growing root bed and minimize mass transfer problems. Indeed, even a hybrid reactor would benefit from biomass immobilization. Adding inoculum into the liquid phase would, after immobilization, allow it to remain distributed at attachment or catch points (Wilson, 1997) and could subsequently be grown further either in a liquid- or gas-phase system. A means of achieving uniform, rapid, and predictable attachment within a bioreactor is therefore desired.

Many studies have focused on cell adhesion to various substrates, including plant cell attachment phenomena (Robins *et al*., 1986; Walach and Pirt, 1986; Facchini *et al*., 1987; Facchini *et al*., 1988; Archambault *et al*., 1989; Facchini, 1990; Facchini and DiCosmo, 1990). Interactions between roots and bacteria, e.g. *Rhizobium*, have been studied for many years (Duverger and Delmotte, 1997; Kijne *et al*., 1997). Interactions between roots and soil have also been discussed (Bowen and Rovira, 1991), but the nature of any bonding between

individual roots and soil particles has received little attention (Fitter, 1996). Even less is known about how roots might adhere to surfaces immersed in liquid, although early work by Tanada (1968) investigated adhesion of root tips of barley (*Hordeum vulgare* L., var *Compana*) and mung bean (*Phaseolus aureus*) to glass. Taken together, these studies suggested that roots have adhesive properties that might be exploited as an immobilization strategy in a bioreactor.

At the pH levels of typical growth media, plant cells have a net negative charge (Facchini *et al*., 1989). Many substrates used for immobilization are also negatively charged, and thus interactions are unfavorable. We investigated various polycations for their ability to reduce the repulsion between plant tissue and substrates without affecting biomass viability. Here we report on the characteristics of the rapid binding of roots to a polypropylene matrix coated with the polycation, poly-L-lysine.

Materials and methods

Plant material and cultivation

Hairy roots of *Artemisia annua* (clone YUT16; Weathers *et al*., 1994) were subcultured every 14 days into 50 mL Gamborg's B5 medium (with 3% (w/v) sucrose, pH 5.7 before autoclaving) in 125 mL shake flasks (Gamborg *et al*., 1968) and incubated on a rotary shaker at 110 rpm and 25±2°C in ambient light (about 5 µmol $m^{-2}sec^{-1}$). Roots grown in the dark at 25±2°C on semi-solid (0.23% w/v Phytagel, Sigma P-8169) B5 medium in petri dishes were inoculated into liquid B5 medium in shake flasks. After 14 days, these roots were transferred to shake flasks for an additional 14 days; subsequently, the tissue was used for experiments.

Hairy roots of *Beta vulgaris* were initiated via the root disk method (DiIorio *et al*., 1992c) using *Agrobacterium rhizogenes* strain ATCC 15834, and were maintained in the dark at 25±2°C on semi-solid B5 medium in petri dishes. A single clone was selected, and inoculum for experiments was obtained from 14 day-old liquid cultures that were inoculated with roots grown on semi-solid medium and then grown as described above for *A. annua* shake flasks.

Normal roots of *Arabidopsis thaliana* (strain Di-3) were obtained by sterilizing seeds according to Reed *et al*. (1998) and then incubating the seeds in liquid B5 medium in shake flasks as described above for *A. annua*. Inoculum for experiments was obtained from plantlets that were subcultured every 14 days.

Biomass measurements

Fresh weights were determined after blotting root tissue on paper towels. Dry weights were obtained after drying the root tissue in a 60°C oven to constant weight.

Binding Test System

All binding studies occurred in 50 mL glass test tubes (21 mm ID, 24 mm OD, 150 mm high). A 2.0 x 3.5 cm piece of polypropylene mesh (R-CMP-1000, Small Parts Inc.) was placed vertically in the bottom of each tube containing 20 mL autoclaved B5 medium (pH 5.7) unless otherwise noted. Each tube contained 20 root segments unless otherwise specified. Root segments were cut 3-4 mm from the root tip and again 3-4 mm behind the first cut (i.e. two segments were cut from each root). Test tubes were incubated in slant racks on a rotary shaker at 110 rpm and 25±2°C in ambient light, and observed. After a 2 hr incubation, roots remaining immobilized on the mesh piece after gentle manual swirling

were considered to be bound. Binding % is defined as [# of root segments attached to mesh] / [total segments] x 100.

Poly-L-lysine coating

Solutions of 0.1% (w/v) poly-L-lysine (P-1274, Sigma Chemical Company) in dH_2O were kept frozen (-20°C) until use. The solution was filter sterilized through a 0.22 µm nylon filter (196-2020, Nalgene) prior to use if necessary. Polypropylene mesh matrices were coated by submersion in the solution for 30 minutes, after which the mesh was air dried.

Antibody interaction protocol

Root segments were incubated in blocking solution (10% (v/v) sheep serum (S-2263, Sigma) in B5 medium) for 60 minutes, and then subjected to three 10-minute rinses in phosphate buffered saline (PBS: 0.14 M NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4, 8.1 mM Na_2HPO_4, pH 7.2; Wang *et al*., 1996) followed by incubation with antibody (sheep anti-human vitronectin purified IgG (SAVN-IG, Enzyme Research Laboratories Inc.), diluted 1/250 in PBS) for two hours, and an additional set of three 10-minute rinses with PBS, before being replaced into the binding test system previously described. All incubations prior to binding assessment occurred in 100 mL glass beakers on a rotary shaker at 110 rpm and 25±2°C in ambient light, with the exception of antibody incubation that was performed in 10 mL glass test tubes to minimize the volume of antibody needed. Previous studies used antibody dilutions from 1/300 to as low as $1/10^4$ (Sanders *et al*. , 1991; Wagner and Matthysse, 1992), thus, we considered that a dilution of 1/250 was adequate for our application.

Protein effects on root binding to mesh and growth

Protein-soaked roots were defined as root segments that were incubated in the following protein solutions diluted with B5 medium for 60 minutes, as if blocking the tissue for antibody studies: 10% (v/v) sheep serum, or an approximate albumin equivalent of 0.6% (w/v) bovine serum albumin (BSA; 9048-46-8, Sigma). To determine the effects of poly-L-lysine on root growth, the amount of poly-L-lysine needed to coat one piece of mesh was measured by weight difference (wet mesh weight minus dry mesh weight, based on an estimated solution density of 1.0 g mL^{-1}). As a worst-case scenario, it was assumed that all of the poly-L-lysine on the mesh went into solution. Roots were inoculated into B5 medium containing the corresponding concentration of poly-L-lysine. Polypropylene mesh was not included. To determine the effects of sheep serum on root growth, roots were either inoculated into 10% (v/v) sheep serum in B5 medium in flasks, or soaked in 10% (v/v) sheep serum for 60 minutes before inoculation into B5 medium; again, no mesh was included with the roots. In a subsequent set of experiments, mesh (both coated and uncoated) was included with the inoculum to assess its effects on root growth.

Trypsinization

After binding to poly-L-lysine coated polypropylene mesh for 2 hours in B5 medium, 2 mL aliquots of trypsin (T-8253, Sigma) were added to give final concentrations of 0.01 or 0.10% (w/v). Control cultures received 2 mL of B5 medium. The number of roots that remained bound to the mesh was measured 60 minutes after trypsin addition.

Lectin interaction with root binding

Four lectins were studied for their ability to block the binding site: concanavalin A (conA, C-7275, Sigma); peanut agglutinin (PNA, L-0881, Sigma); wheat germ agglutinin

(WGA, L-9640, Sigma); and *Ricinus communis* agglutinin I (RCA-I, L-7886, Sigma). Lectins were dissolved or diluted in dH_2O at a concentration of 100 μg mL^{-1}; this concentration was sufficient to block nodulation of *Macroptilium atropurpureum* by *Rhizobium* (Ridge and Rolf, 1986). Uncut roots (1.0 g FW) were incubated in 10 mL of solution for 30 minutes, and then segments were cut from the roots and inoculated into the binding test system. In a separate experiment, root segments were incubated with various concentrations of concanavalin A for 60 minutes prior to addition of poly-L-lysine coated polypropylene mesh. Sharon and Lis (1989) reported that conA requires Mn^{2+} and Ca^{2+} in order to be active; therefore, these cations were added to the incubation medium as $MnCl_2 \cdot 4H_2O$ (M-8530, Sigma) and $CaCl_2 \cdot 2H_2O$ (C-2356, Sigma), respectively. Subsequent studies examined the individual effects of both divalent cations as well as that of Mg^{2+} (as $MgCl_2 \cdot 6H_2O$, M-2393, Sigma) on root binding.

Statistical analysis

Standard deviations from the mean were calculated for each experiment. Unpaired student t-tests were used to determine statistical significance at P=0.05.

Results and discussion

We undertook a study of root binding to inert substrates because we had noticed that roots often became attached to walls of glass shake flasks and plastic reactor vessels. Based on preliminary studies of a number of mesh substrates (neoprene, nylon, polyester, polypropylene, stainless steel) and polycations (cetylpyridinium chloride, chitosan, hexadimethrine bromide, polyethylenimine, poly-L-lysine, protamine), we chose to

further investigate the use of poly-L-lysine coated polypropylene mesh for immobilization of roots.

General binding features

Microscopic examination of *A. annua* root segments bound to the mesh revealed that the roots were often bound to the polypropylene strands via extended root hairs (Figure 1), although adhesion also occurred directly with hairless epidermal cells. The attached root hairs seemed to function like elastic tethers. Roots attached by root hairs would bounce back and forth when gently shaken, but then returned to their original tethered position. Approximately 80% of the *A. annua* hairy root segments became bound to poly-L-lysine coated polypropylene mesh within a few minutes after addition to the test systems, and all binding was complete within two hours (data not shown). To determine if this binding was unique to *A. annua* or more broadly applicable to other species, we also compared *Beta vulgaris* transformed with the same strain *of Agrobacterium rhizogenes* and a nontransformed species, *Arabidopsis thaliana*. The roots of all three species responded similarly in that all showed a significant increase in the level of binding achieved when the supporting matrix was coated with poly-L-lysine: *A. annua*, 81%; *B. vulgaris*, 48%; *A. thaliana*, 90%. For all three species, the process was complete within minutes (data not shown). These results suggest that the binding process is common to a variety of species, and that it is not necessarily influenced by *Agrobacterium* transformation. Of the three species tested, *Beta vulgaris* hairy roots consistently showed lower levels of binding, possibly because poly-L-lysine also affects cell membranes and causes pigment efflux from beet root tissue (Siegel and Daly, 1966). Pigment levels in the medium were not analyzed,

nor was growth monitored in the experiments reported here; however, no visible coloration of the medium (indicative of pigment leakage) was noted.

Proteins involved in binding

In an effort to understand the binding process, we searched for adhesion molecules, including plant vitronectins and glycoproteins, in the roots that might be common to a number of species. We hypothesized that if these molecules were blocked through use of specific compounds, and subsequent adhesion did not occur, it was likely that a similar molecule was involved in the mechanism of root attachment to poly-L-lysine coated polypropylene mesh.

Plant adhesion molecules similar to human vitronectins are common in many plants (Sanders *et al*., 1991; Wagner and Matthysse, 1992; Wagner *et al*., 1992; Zhu *et al*., 1993; Zhu *et al*., 1994; Wang *et al*., 1994). A vitronectin-like molecule, however, is probably not involved in the process of root attachment for *A. annua, B. vulgaris*, or *A. thaliana* because binding occurred despite the addition of vitronectin antibodies to the binding test system (data not shown).

Plant cells are also known to have a diversity of polysaccharides, proteins, and glycoproteins associated with their cell surface (Kolster and Biederbeck, 1987), and use of lectins can provide useful information about binding sites. To test whether glycoproteins may be involved in binding we used four lectins: concanavalin A (conA, specific for α-L-mannose/α-L-glucose), peanut agglutinin (PNA, specific for D-galactose-β(1,3)N-acetyl-α-D-galactosamine), wheat germ agglutinin (WGA, specific for sialic acid), and *Ricinus communis* agglutinin I (RCA-I, specific for β-D-galactose). However, none of the four

lectins inhibited adhesion of *A. annua* roots to the poly-L-lysine coated mesh, suggesting that glycoproteins are not involved in the adhesion process (data not shown).

Mammalian cells readily adhere to surfaces coated with poly-L-lysine, and can be rapidly detached using trypsin. We trypsinized attached roots for 60 minutes, but no detachment of roots occurred (data not shown). Kolster and Biederbeck (1987) similarly showed that various enzymes were ineffective in detaching cells of *Calystegia sepium* L. that were bound to various substrates including glass, polystyrene, polyethylene-tetraphthalene, and aluminum foil.

Protein effects on root growth

If poly-L-lysine is to be used to immobilize roots in a bioreactor, it is important to determine whether subsequent growth is affected. Poly-L-lysine did not significantly increase root biomass compared to the B5 control, although the DW/FW ratio did increase slightly (Table 1). These data also showed that the amount of poly-L-lysine used to coat the mesh was not toxic to *A. annua* hairy roots (Table 1). The amount of poly-L-lysine used in this experiment, 0.2% (w/v), was an amount estimated to be coating the mesh. Roots cultivated with poly-L-lysine coated mesh, with or without a prior incubation in sheep serum, showed increased growth compared to controls grown with uncoated mesh (Table 2). DW/FW ratios were, however, statistically identical for all conditions.

Interestingly, the sheep serum used in the vitronectin-antibody experiments appeared to increase the number of *A. annua* roots that bound to poly-L-lysine coated mesh, and a subsequent experiment confirmed that incubating roots of *A. annua* in sheep serum enhanced the binding process by about 50%. This effect was not a general protein effect, however, because roots soaked in a solution of BSA were not similarly stimulated.

To determine whether the sheep serum created an artifact in antibody binding studies by affecting root growth, roots were incubated in B5 medium containing sheep serum and growth was measured after 14 days. Continuous culture in 10% (v/v) sheep serum inhibited root growth and promoted visual callus formation (as evidenced also by the higher DW/FW ratio, Table 1). In contrast, the short (60 min) incubation period in sheep serum used prior to the vitronectin binding study, resulted in growth equivalent to that of roots grown in B5 medium, although the DW/FW ratio was slightly higher (Table 1). Whereas BSA is a single pure protein, sheep serum is a complex mixture of proteins and other small molecules. It is thus possible that any one of these other compounds affects the stimulation of binding by sheep serum.

Immobilization effects on root growth

To determine whether the physical process of immobilization stimulated root growth, roots were grown without mesh and compared to those grown with uncoated or poly-L-lysine coated mesh. There was no significant increase in growth in the presence of mesh (Table 2). Although there was a small but significant increase in DW, thereby also altering the DW/FW ratios, this increase may have been due to a slight increase in callus formation in root mats entangled in the mesh pieces. Taken together, these data suggest that there are no measurable effects on growth from either the addition of poly-L-lysine or the physical process of immobilization of roots on mesh.

Divalent cations affect binding

During our studies with the lectin conA, we observed that the required "activating" cations, Mn^{2+} and Ca^{2+}, significantly decreased *A. annua* root binding. To investigate this further, subsequent experiments involved the addition or exclusion of 3 divalent

cations (Mn^{2+}, Ca^{2+}, and Mg^{2+}) from the binding solution. The data in Table 3 show that only Mn^{2+} appeared to affect binding. Increased levels of Mn^{2+} resulted in a significant decrease in binding. Studies with various concentrations of Mn^{2+} indicated that this effect is not apparent until at least 5 mM excess Mn^{2+} is present (data not shown). The basal levels of Mn^{2+}, Ca^{2+}, and Mg^{2+} in B5 medium are 0.059, 1.02, and 1.01 mM, respectively. For Mn^{2+}, 5 mM represents a nearly 100-fold increase in concentration. Although excess Mn^{2+} can inhibit attachment, once the roots are bound to the mesh, addition of 10 mM excess Mn^{2+} did not result in detachment (data not shown).

In many instances, the physiological state of the cell/organism has a significant effect on its adhesion properties (Van Haecht *et al*., 1984; Facchini *et al*., 1988; Archambault *et al*., 1989). Consequently, we considered that the large increase in Mn^{2+} concentration may have affected the metabolism of the roots. When roots were grown in B5 medium plus 10 mM Mn^{2+} for 14 days, there was very little increase in biomass. However, if roots were incubated in this medium for 2 hours and then transferred into normal B5 medium, growth was comparable to controls (data not shown). The cations Ca^{2+} and Mg^{2+} were not tested for their effects on growth because they had not been shown to affect the binding process. Although it is not clear why Mn^{2+} and not the other divalent cations tested affected the level of attachment of *A. annua* roots to the mesh, others have observed some species and substrate specificity of cations in adhesive responses (Walach and Pirt, 1986; Facchini *et al*., 1989). For example, Na^{+} had no effect on the adhesion *of Catharanthus roseus* cells to several plastic substrates, whereas Ca^{2+} slightly stimulated adhesion; and Al^{3+} increased adhesion up to a concentration of 100 mM, after which adhesion decreased (Facchini *et al*., 1989). For the aforementioned studies, the plastic substrates were not coated with poly-L-

lysine and therefore were negatively charged. In studies using *Chlorella*, Walach and Pirt (1986) observed that the cells bound more efficiently to glass when grown in a modified medium, and in contrast to our results, a decreased level of magnesium decreased the level of cell adhesion. Reducing the concentration of iron, calcium, and trace elements resulted in an increased level of adhesion; however, these components tested individually showed little effect on binding of *Chlorella*.

Other factors and binding

In an effort to reduce variability in the percentage of roots binding to poly-L-lysine coated polypropylene mesh for a given set of conditions, a number of other factors were tested. There was no significant difference in binding for root tips versus the adjacent segment (data not shown). Although *A. annua* roots grow better in filter sterilized medium than in autoclaved medium (unpublished data), adhesion was not affected. While Facchini *et al*. (1989) and Mozes *et al*. (1987) found that the pH of the suspending medium had a measurable effect on the adhesion of *Catharanthus roseus* cells and *Moniliella pollinis*, respectively, our experiments showed that the level of binding of *A. annua* roots was not affected in a pH range of 4.0-8.0. Furthermore, the ionic strength of the phosphate buffer used (0.01 mM to 1.0 mM) did not appear to affect binding. Allowing the root segments a period of acclimatization (0 to 60 minutes) prior to the addition of mesh, and varying the time period between mesh addition and observation of binding (1 to 2 hours) also had no significant effect on binding measurements (data not shown).

Conclusions

We have demonstrated that roots of three different species, *Artemisia annua*, *Beta vulgaris*, and *Arabidopsis thaliana*, will adhere to a poly-L-lysine coated polypropylene substrate. Roots attach to the substrate within minutes, and do not adhere to polypropylene without the poly-L-lysine coating. The attachment phenomenon apparently does not involve either vitronectin-like molecules or glycoproteins. The amount of poly-L-lysine used in these studies was not toxic to roots of *A. annua*. Although the divalent cation Mn^{2+} added in excess decreased binding, Ca^{2+} or Mg^{2+} did not. However, once roots were attached to the substrate, addition of Mn^{2+} did not effect detachment. The excess Mn^{2+} was not detrimental to root growth after short-term exposure, but growth was arrested in continuous culture. Attachment of roots does not appear to be affected by the method of media sterilization, pH, ionic strength, acclimatization period in binding solution, or length of incubation prior to binding measurements. Adhered roots did not detach after trypsinization. These results show that roots can be bound quickly and in a controlled manner to an inert substrate. This information will prove useful not only for the immobilization of hairy roots in bioreactors, but also in furthering the understanding of the fundamental biology of roots.

Table 2.1. Effect of poly-L-lysine and sheep serum on *A. annua* root growth.

CONDITION	$\frac{FW_f - FW_o}{FW_o}$ after 14 d	$\frac{DW_f - DW_o}{DW_o}$ after 14 d	$\frac{DW}{FW}$ ratio ($\times 10^{-2}$)
control (B5)	6.3±0.69 *a*	6.2±0.58 *a*	7.5±0.14 *a*
0.2% (w/v) poly-L-lysine in B5	5.9±0.64 *a*	6.1±0.53 *a*	7.8±0.15 *b*
10% (v/v) sheep serum in B5	2.2±0.33 *b*	3.5±0.54 *b*	10.8±0.23 *c*
60' 10% sheep serum soak, then grown in B5	6.4±0.62 *a*	6.7±0.49 *a*	8.1±0.18 *b*

Inoculum was 0.30 g blotted fresh weight of *A. annua* hairy roots in 125 mL shake flasks containing 50 mL B5 medium. There were at least 4 replicates per condition tested. Cultures were incubated at 110 rpm and 25±2°C in ambient light for 14 days. Letters indicate statistical significance within a column at P = 0.05.

Table 2.2. Effect of mesh, poly-L-lysine coating, and sheep serum soaking on *A. annua* root growth.

CONDITION	$\frac{FW_f - FW_o}{FW_o}$ after 14 d	$\frac{DW_f - DW_o}{DW_o}$ after 14 d	$\frac{DW}{FW}$ ratio ($\times 10^{-2}$)
uncoated mesh + roots	6.7±0.27 *a*	6.8±0.24 *a*	7.8±0.12 *a*
poly-L-lysine coated mesh + roots	7.7±0.43 *b*	7.5±0.31 *b*	7.5±0.17 *a*
poly-L-lysine coated mesh + sheep serum pre-soaked roots	8.3±0.22 *b*	8.3±0.19 *c*	7.7±0.05 *a*
no mesh	7.0±0.27 *a*	4.7±0.21 *a*	7.1±0.13 *a*
uncoated mesh + roots	7.1±0.70 *a*	5.2±0.25 *b*	7.6±0.34 *b*
poly-L-lysine coated mesh + roots	7.3±1.12 *a*	5.3±0.67 *a,b*	7.6±0.30 *b*

Inoculum was 0.30 g blotted fresh weight of *A. annua* hairy roots in 125 mL shake flasks containing 50 mL B5 medium. There were at least 4 replicates per condition tested. Cultures were incubated at 110 rpm and 25±2°C in ambient light for 14 days. Break in table indicates separate experiments. Letters indicate statistical significance within a column and for each separate experiment at P = 0.05.

Table 2.3. Effect of Mn^{2+}, Ca^{2+}, and Mg^{2+} on binding of *A. annua* root segments to poly-L-lysine coated polypropylene mesh.

$MnCl_2$ (mM)	$CaCl_2$ (mM)	$MgCl_2$ (mM)	% BOUND
0.059	1.02	1.01	79 *a*
10.059	1.02	1.01	55 *b*
0.0	1.02	1.01	85 *a*
0.059	11.02	1.01	73 *a*
0.059	0.0	1.01	78 *a*
0.059	1.02	11.01	71 *a*
0.059	1.02	0.0	85 *a*

A total of 20 root segments for each condition was inoculated into the indicated test solutions, and there were at least 4 replicates per condition tested. Measurements were taken 2 hours after addition of mesh. Letters indicate statistical significance at P = 0.05

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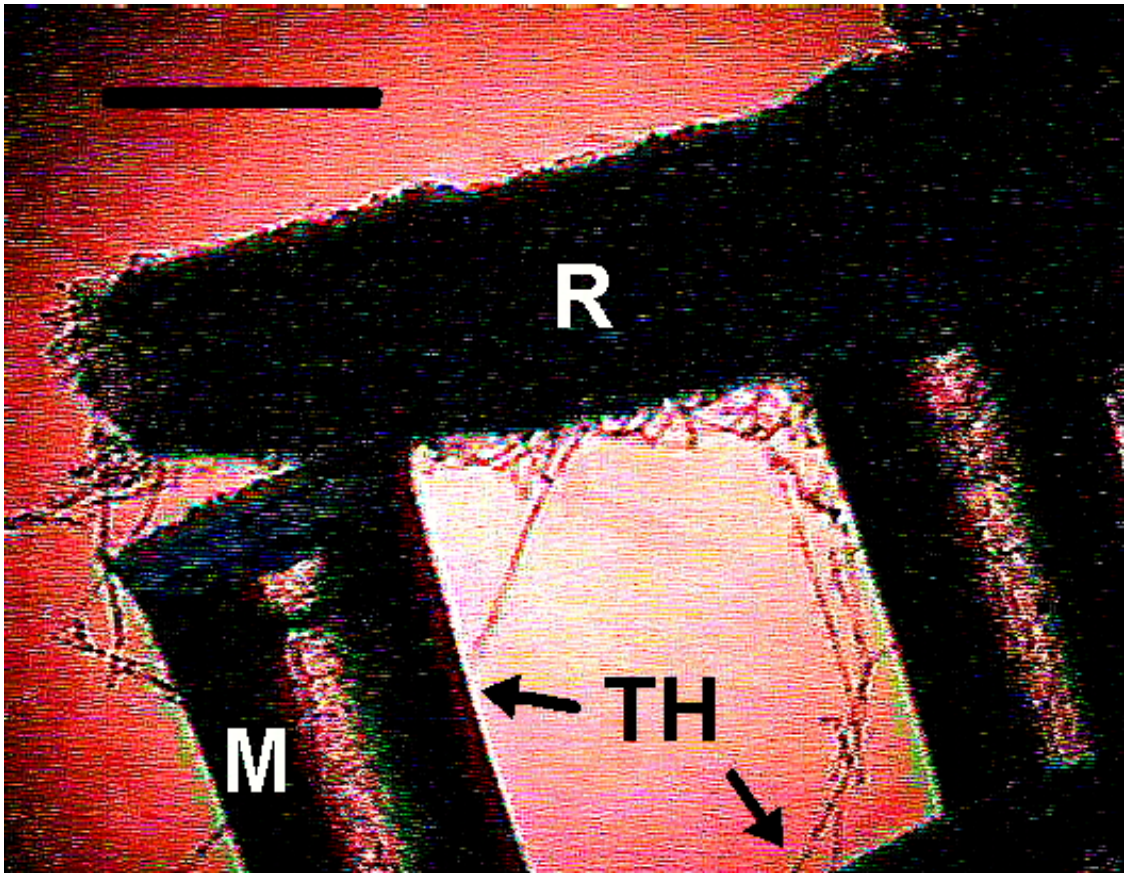

Figure 2.1. Root fragment with extended root hairs bound to poly-L-lysine coated polypropylene mesh. R, root; M, polypropylene mesh; TH, tethered root hairs. Bar = 500 µm.

3

Effect of biomass density, sucrose concentration, and feed rate on growth and biomass yield of transformed roots of *Artemisia annua* in a nutrient mist bioreactor

Introduction

The design of a bioreactor for growth of hairy roots must take into consideration the morphological characteristics of the tissue. The mist reactor reduces the gas-exchange limitations and shear conditions normally found in liquid-phase reactors. It should be noted that successful establishment of a hairy root culture on solid medium does not guarantee that these roots will continue to grow when transferred to liquid medium (Hallard et al., 1997), so a gas-phase reactor may be a necessary option in those instances. Oxygen is essential for respiration and thus, growth of roots. The ability to attain a high biomass density is an important part of an economically viable production scheme, and the maximum root tissue concentration that can be achieved depends on the delivery of oxygen and other nutrients into the dense matrix (Curtis, 2000). According to studies by McKelvey et al. (1993), roots are more capable of compensating for poor liquid dispersion than for poor gas dispersion within reactor systems (Curtis, 1993). To assess the response of hairy roots to altered levels of oxygen in mist reactors, alcohol dehydrogenase (ADH) mRNA was measured in *A. annua* hairy roots. Comparison of ADH mRNA expression in both shake flasks and bubble column reactors to mist reactors showed that mist-grown roots were not oxygen limited (Weathers et al., 1999). There

was no detectable expression of ADH mRNA in roots grown in the mist reactor to a density of about 37% (v/v) (Kim, 2001). This is equivalent to a packing fraction (α, which equals the fresh weight concentration (g L^{-1}) × 0.001) of 0.37. However, expression was detected in roots from the bubble column at packing densities as low as 6% (v/v; α=0.06) (Weathers et al., 1999).

The respiratory quotient, which is the ratio of CO_2 produced to O_2 consumed, increases with the degree of anaerobic respiration (Bordonaro and Curtis, 2000). As previously stated, *A. annua* roots are oxygen-deprived in a liquid-phase bubble column reactor (Weathers et al., 1999) and thus are not functioning at their fullest aerobic capacity. When Bordonaro and Curtis (2000) compared the respiratory quotients of *Hyoscyamus muticus* hairy roots grown in either a bubble column reactor or a trickle bed reactor and noted that the former was consistently higher, it suggested that anaerobic respiration was occurring in the liquid-phase system. The respiratory quotient in the mist reactor system described herein could not be measured accurately because the intermittent air flow used did not allow the roots to reach a steady state of respiration, but based on the lack of metabolic oxygen-stress indicators and the highly aerated environment in the mist reactor, the roots should grow more efficiently compared to liquid-phase systems. Ultimately, if a profitable method of root culture is desired, not only must the amount of biomass produced per unit volume be maximized, but the conversion of costly nutrients into that biomass should be optimized as well.

Kim et al. (2002a) suggested that in a mist reactor, gas dispersion is improved at the expense of liquid dispersion; that is, availability of nutrients in the liquid phase may be an issue. Roots grown in a bubble column reactor had higher dry mass compared to

those harvested from the mist reactor. This unexpected result was explained through modeling of mist deposition dynamics (Kim et al., 2002a). If an adequate supply of nutrients does not reach the surface of all roots due to insufficient droplet transport and deposition, growth may be limited. Mist deposition is consequently a crucial step in the mass transfer of nutrients to the roots in a mist reactor (Wyslouzil et al., 1997). Application of the standard aerosol deposition model for fibrous filters to mist deposition in hairy root beds was described by Wyslouzil et al. (1997). We hypothesized that there were several possible approaches to improve the growth rate in a mist reactor. Increasing the initial biomass density should increase the efficiency of nutrient mist particle capture by the roots. Increasing the nutrient concentration should increase the growth rate since the volume of medium required, and thus the amount of mist the roots must capture, would be lower. Increasing the medium delivery rate should also increase the amount of nutrients available to the roots, though there are additional issues concerning the alteration of misting cycles that will be addressed later.

We studied the effect of packing fraction on growth rate by reducing the size of the mist reactor growth chamber from 1.5 L to 0.05 L and inoculating with roots at packing fractions of α=0.19-0.39. Growth rates were measured and compared at different sucrose concentrations and misting cycles in order to examine the effects of nutrient concentration and feed rate. Since it has already been determined that high biomass densities of tissue can be grown in the mist reactor without oxygen deprivation, if we can also improve the growth rate such that it meets or exceeds that achieved in conventional liquid-phase reactors, it will be a very important step toward promoting commercial feasibility of this unique system.

Materials and methods

Cultures and their maintenance

Transformed roots of *A. annua* (clone YUT16; Weathers et al., 1994) were maintained on petri dishes containing semi-solid media (Gamborg's B5 medium (Gamborg et al., 1968) with 3% w/v sucrose and 0.23% w/v Phytagel). The medium was adjusted to pH 5.7 before autoclave sterilization, and plates were incubated in the dark at 23±2°C. All inoculum for experiments was obtained from cultures that were first subcultured from plates to liquid culture (125 mL shake flasks containing 50 mL Gamborg's B5 medium with 3% w/v sucrose) for two passages. Flasks were placed on a shaker at 100 rpm, 23±2°C, under continuous ambient cool white fluorescent light (~4 μE m^{-2} s^{-1}). Inoculum used for experiments was 14-15 days old, and all media were filter sterilized (0.22 μm) to eliminate variation in initial sugar profiles from thermally degraded monosaccharides during autoclaving (Weathers et al., 2004).

Mist reactor culture

The nutrient mist bioreactor described by Weathers et al. (1999) was modified (Fig. 3.1) for these studies and is henceforth referred to as the differential mist reactor. The 1.5 L growth chamber was replaced with a much smaller (~45 mL volume, ~30 mm diameter) cylinder fabricated from a 50 mL polypropylene conical centrifuge tube. The misting cycle was 15 min on / 15 min off unless noted otherwise and was controlled using a timer (GraLab timer 541, Dimco-Gray Co., Centerville, OH). The air flow rate was 4 L min^{-1} during the "on" cycle and was supplied by a vacuum pressure pump (400-1901, Barnant Co., Barrington, IL). This cycle delivered 0.88 mL min^{-1} of liquid nutrients. Air was humidified using a MH single-tube Nafion humidifier (Perma Pure,

Inc., Toms River, NJ) before passage through a sterile 0.22 μm filter. Roots harvested from shake flasks were blotted and weighed prior to manual inoculation into the growth cylinder at the desired initial packing fraction (α, which equals the fresh weight concentration (g L^{-1}) × 0.001). The biomass/medium volume ratio was kept constant at 8 g FW L^{-1} (0.52 g DW L^{-1}) in both mist reactor and shake flask studies.

Biomass measurements

Roots were harvested after 6 days of growth in the differential mist reactor for packing fraction experiments, or on days 2, 4, and 6 for growth kinetics studies. A few mist reactor experiments were run longer (10-15 days). Shake flasks were harvested on day 14 for sucrose concentration experiments, and on days 2, 4, 6, 9, 12, and 14 for growth kinetics studies. Roots were rinsed with distilled water and blotted on paper towels to determine fresh weight, and then dried in a 60°C oven to determine dry weight.

The average specific growth rate (μ_{avg}) was calculated as:

$$\mu_{avg} = \frac{\ln \frac{DW_f}{DW_i}}{\Delta t} \qquad [3.1]$$

where subscripts f and i denote final and initial dry weights, respectively, and units of time (t) are days.

The apparent biomass yield ($Y_{X/S}$) was calculated as the ratio of biomass increase to total sugar consumption:

$$Y_{X/S} = \Delta \text{ g DW} / \Delta \text{ g glucose equivalent} \qquad [3.2]$$

where g glucose equivalent = g glucose + g fructose + (360/342) × g sucrose. The factor of (360/342) takes into account the molecule of water that is incorporated during

hydrolysis of sucrose (Sharp and Doran, 1990). The total remaining volume of medium in the reservoir and tubing (or shake flask as appropriate) was measured, and an aliquot was stored at –20°C until residual nutrients were analyzed.

Media analyses

Sucrose, glucose, and fructose concentrations were measured via HPLC (Waters, Milford, MA) using a Microsorb amino column (5 μm, 250 × 4.6 mm, 100 Å, Varian Analytical Instruments, Walnut Creek, CA) with a R401 refractive index detector (Waters, Milford, MA) and a mobile phase of 85:15 v/v acetonitrile:water at a flow rate of 1 mL min^{-1}. Fructose, glucose, and sucrose were used as standards. Nitrate concentrations were analyzed spectrophotometrically as described in Kim et al. (2003). Standard solutions were made from KNO_3. Phosphate concentrations were measured spectrophotometrically by mixing 100 μL sample with 3 mL working phosphorus reagent and then reading the absorbance at 650 nm after 10 minutes. Working phosphorus reagent recipe is as follows: 0.472 g ammonium molybdate were dissolved in 118 mL of 80 mL L^{-1} sulfuric acid. This solution was added to 118 mL of 20 g L^{-1} polyvinylpyrrolidone (Sigma P-2307). Ferrous ammonium sulfate (0.96 g) was then added to the combined solutions. Standard solutions were made from $NaH_2PO_4 \cdot H_2O$. Specific conductivity of the medium was measured using a conductivity meter (Cole-Palmer, Vernon Hills, IL).

Data analyses

Unpaired student t-tests were used to determine statistical significance between various experimental conditions. There was a minimum of 3 replicates in each case, except where noted otherwise.

Results and discussion

Increased packing fraction increases growth rate

The mist deposition model (Wyslouzil et al., 1997) shows that dense beds of roots can be compared to fibrous filters. The ideal filter has evenly distributed fibers that lie perpendicular to the flow. Though root beds have regions of high and low packing density and growth occurs in all directions, the qualitative trends and behavior of mist deposition can still be studied by applying the model. When the model was tested on root beds that had been manually packed to $\alpha = 0.5$, it was found to correspond well to experimental data as long as the Reynolds number (Re) was < 10 (Wyslouzil et al., 1997). In this system, 5.29 < Re < 9.93. The Reynolds number characterizes the relative importance of inertial and viscous forces, and for filtration problems:

$$\mathrm{Re} = \frac{\rho U_o D_R}{\mu_g} \qquad [3.3]$$

where ρ and μ_g are the density and viscosity of the carrier gas, D_R is the diameter of the root, and U_o is the gas velocity in the root bed. In terms of the number of droplets captured, the efficiency (η_B) of the root bed is a function of the particle diameter (D_P) and is equal to:

$$\eta_B = 1 - \exp\left[\frac{-4L\alpha\eta_C}{D_R(1-\alpha)}\right] \qquad [3.4]$$

where L is the length of the root bed and:

$$\eta_C = 1 - (1 - \eta_{IMP+INT}) \times (1 - \eta_D), \qquad [3.5]$$

the combined capture efficiency (η_C) due to impaction and interception ($\eta_{IMP+INT}$), and diffusion (η_D). Determining $\eta_{IMP+INT}$ involves solving two nonlinear equations as found

in Crawford (1976), and the expression for calculating η_D is given in Friedlander (1977). The equations for $\eta_{IMP+INT}$ are also given in Kim et al. (2002a). The overall mass deposition efficiency (η_{OM}) of the root bed is the product of the root bed efficiency $\eta_B(D_{Pi})$ and the mass fraction $m(D_{Pi})$ of mist particles of diameter D_{Pi} summed over the aerosol size distribution data:

$$\eta_{OM} = \sum_i \eta_B(D_{Pi}) \times m(D_{Pi}) . \qquad [3.6]$$

The amount of medium captured by the roots (V_{dep}) in mL per day is expressed as:

$$V_{dep} = 24\omega \times Q_L \times \eta_{OM} \qquad [3.7]$$

where 24 is the conversion factor from hours to days, ω is the duty cycle in minutes per hour, and Q_L is the medium flow rate in mL per minute during the mist "on" cycle.

The amount of medium required to support the growth of roots (V_{req}) in mL per day depends on: the density of the roots ρ_{FW} (grams fresh weight per mL), the dry weight / fresh weight ratio (*DW/FW*), the specific growth rate μ (day^{-1}), the nutrient concentration in the medium C_S (g L^{-1}), the apparent biomass yield of the growth-limiting nutrient $Y_{X/S}$ (g DW biomass per g nutrient consumed), the working volume of the reactor V (L), and packing fraction α. The expression for V_{req} is:

$$V_{req} = 10^6 \rho_{FW} \times \frac{DW}{FW} \times \frac{\mu}{C_S} \times \frac{1}{Y_{X/S}} \times V \times \alpha \qquad [3.8]$$

Obviously, V_{dep} must be equal to or greater than V_{req} in order to maintain a desired growth rate μ.

Insufficient nutrient availability was proposed as a possible explanation for the apparent growth limitations of *A. annua* hairy roots in the nutrient mist bioreactor (Kim

et al., 2002a). Since V_{dep} is a function of the packing fraction (α), increasing α should increase V_{dep} and thus support a higher growth rate by allowing more nutrients to be captured by the roots. V_{req} also depends on α, but it does so in a linear manner, while a plot of V_{dep} versus α yields a sigmoidal curve. Though values will vary depending on the growth rate, nutrient concentration, and root diameter, generally speaking, $V_{dep} > V_{req}$ at higher values of α. When mist mode commenced at packing fractions that were at most 0.056, Kim et al. (2002a) observed an average specific growth rate (μ) of 0.07 day^{-1}. In contrast, the average specific growth rate in the differential mist reactor at initial packing fractions ranging from 0.193-0.387 was 0.12 day^{-1} for a 6-day period (Fig. 3.2). Direct comparison between these systems, however, is complicated due to different culture times and operating conditions. Nevertheless, it appears that roots grown in the differential mist reactor had higher growth rates than those observed by Kim et al. (2002a), supporting the hypothesis that a higher initial inoculum density positively affects subsequent growth in mist reactors. It was not possible to manually pack the roots to $\alpha <$ 0.193 since the roots had a natural tendency to compact themselves to this minimum density in the growth chamber. Since there was no statistical difference between the three packing fractions tested, the intermediate value ($\alpha = 0.29$) was used in subsequent experiments. As a comparison, roots were inoculated into a shake flask at the same packing fraction (14.5 g FW in 50 mL B5 medium with 3% sucrose), and the growth rate after 6 days was only 0.01 day^{-1} (Table 3.1). If we assume an apparent biomass yield of 0.35 g DW produced per g sugar consumed, the highest possible biomass increase would be 0.55 g DW, which corresponds to a growth rate of 0.08 day^{-1}. Since this was not achieved, one can infer a combination of liquid (nutrient) and gas (oxygen) mass transfer

limitation into the densely inoculated roots, which were packed at a level approximately 30× higher than that used for experiments using shake flasks.

In one mist reactor experiment, roots were allowed to grow from an initial packing fraction of 0.39 in the differential mist reactor for 10.3 days. A final packing fraction of 0.71 was achieved with a growth rate of 0.11 day^{-1}, and the harvested roots appeared healthy and non-necrotic throughout the dense bed. This density of biomass is extremely difficult to achieve in a liquid-phase system. At high tissue concentrations (10-40 g DW L^{-1}, or a packing fraction range of about 0.20-0.80), the high fluid flow resistance of the root beds limits the ability to scale-up submerged culture reactors (Carvalho and Curtis, 1998). *Hyoscyamus muticus* hairy roots grown in a 2 L convective flow bioreactor reached a final fresh weight density of 556 g L^{-1} ($\alpha = 0.56$) after one month. Oxygen was supplied via external aeration of the medium, which was then forced through the root bed using a positive displacement pump. The power required to overcome the flow resistance and maintain a minimum oxygen concentration in the 2 L reactor at the time of harvest was estimated as 2 W L^{-1}, which is comparable to a typical agitator power requirement for microbial fermentation (Carvalho and Curtis, 1998). However, while one can approximate scale-up conditions by assuming constant power per unit volume in a well-mixed microbial fermentation, the power required to maintain a given flow rate increases as the length and/or width of the root bed increases due to the higher pressure drop across the bed, and scale-up becomes unfeasible. The flow resistance of a highly packed root bed is similar to a column of packed sand (Nield and Bejan, 1991).

A final fresh weight density of 753 g L^{-1} ($\alpha = 0.75$) was attained by Ramakrishnan and Curtis (2004) for hairy roots of *H. muticus* in a 14 L trickle bed reactor with oxygen

enrichment and fed-batch additions of fresh medium. The growth rate was 0.21 day^{-1}, or 0.13 day^{-1} without oxygen supplementation. In a scaled-down (1.6 L) version of that reactor, Ramakrishnan and Curtis (2004) achieved a growth rate of 0.35-0.40 day^{-1} at the initiation of trickle bed operation, but it then steadily declined to less than 0.10 day^{-1} after only 4 days. We were able to attain a comparable biomass density (as high as $\alpha = 0.71$) and growth rate in our system without supplementation of the growth medium, and oxygen was supplied from ambient room air. That said, our mist reactor should be considered a "differential" reactor (McKelvey et al., 1993), and the biomass/medium ratio was about 8× higher and the bed depth was about 5× deeper in the 1.6 L trickle bed reactor than in the our mist reactor. Any performance comparisons must take this into account.

Increased carbon concentration increases growth rate

V_{req} (the volume of media required by the roots to grow at a particular growth rate) is inversely proportional to the nutrient concentration C_S; therefore, increasing C_S should decrease V_{req}. The major nutrients in B5 medium are sugar, nitrogen-containing compounds (nitrate and ammonium), and phosphate. Here we chose to alter sucrose concentration. Using the differential mist reactor system previously described, *A. annua* hairy roots were fed medium containing 3% or 5% sucrose. After 6 days, roots grown with 5% sucrose had a significantly higher specific growth rate (see Fig. 3.3; 0.18 day^{-1} for 5% sucrose versus 0.12 day^{-1} for 3% sucrose). When the growth kinetics of roots grown in 3% or 5% sucrose (Fig. 3.3) are examined, it appears that the highest growth rate occurs by day 4 at both sugar levels. At day 6 in 5% sucrose, the growth rate is equivalent to that in shake flasks (Table 3.1). Although the growth rate actually

decreased (Fig. 3.3) from days 4-6 for roots grown in 3% sucrose, carbon (measured as sugar; Fig. 3.4) was not depleted in this brief time period. Analysis of other medium constituents is discussed later. While the reasons for the slower growth at this early stage are not known, the results suggest that a high sucrose concentration needs to be maintained both to achieve and to sustain a higher growth rate in the mist reactor. A similar trend in growth was seen in shake flasks when roots were grown on 5% sucrose (Fig. 3.5). There was a noticeable lag phase for roots grown on 3% sucrose, and growth decrease was evident after day 9, although sugar was not exhausted (see Fig. 3.9 for residual sugar data).

Increased misting does not significantly increase growth rate

Lengthening the duration of the misting cycle would appear to be an obvious way of increasing the amount of nutrients delivered to the roots and thereby increase growth, but this solution is actually not so simple. For reasons not fully understood, the misting cycle plays a significant role in growth of roots in a mist bioreactor (Table 3.2). Liu et al. (1999) found that a misting cycle of 3 min on / 30 min off was the optimum of those tested for transformed roots of *A. annua* grown in their nutrient mist bioreactor, though its design and operating conditions were different than those implemented by Weathers et al. (1999). Liu et al. (1999) provided gas either when mist was not being generated, or continuously; while Weathers et al. (1999) provided gas only when the mist was being generated. Additionally, Liu et al. (1999) fed mist to the bottom of the growth chamber, whereas Weathers et al. (1999) fed mist from the top. Co-current down-flow of gas and liquid phases, along with gravity, facilitates drainage in mist reactors that are top-fed. Reactors fed from the bottom encourage liquid accumulation in lower layers of tissue,

particularly when the bed has become dense. Indeed, Liu et al. (1999) noted a greater than 50% decrease in biomass between the bottom and top layers. Feeding from the bottom can not only inhibit mist from reaching the upper layers, but if a significant amount of liquid becomes entrapped in the lower layers, that section of tissue becomes a liquid- rather than a gas-phase culture. Chatterjee et al. (1997) found that a misting cycle of 1 min on / 15 min off caused transformed roots of *A. annua* to darken and become necrotic after 12 d. As in Liu et al. (1999), this system was also fed mist from the bottom.

Studies with hairy roots of *Beta vulgaris* and *Carthamus tinctorius* showed that either increasing or decreasing the "off" time beyond a certain point adversely affected root growth of those species (DiIorio et al., 1992b). Yet, studies of single transformed roots of *A. annua* (Wyslouzil et al., 2000) showed that a misting cycle of 1 min on / 15 min off promoted healthier-looking roots and higher fresh final biomass yields versus the other cycles tested (5 min on / 15 min off and 1 min on / 60 min off). In our studies, the misting cycle was modified so that the total amount of sucrose fed was maintained while the sucrose concentration was varied. Figure 3.6 compares the growth rates of roots grown with equivalent hourly sucrose feed rates under different misting cycles. Although a misting cycle of 15/3 with 1.8% sucrose provided the same amount of carbon per hour as a misting cycle of 15/15 with 3% sucrose, growth was significantly different. Similar results were observed with a misting cycle of 15/3 with 3% sucrose, which was equivalent to a 15/15 misting cycle with 5% sucrose. Growth was significantly different (Fig. 3.6). Growth was essentially the same for 3% sucrose at two different misting cycles. Taken together, these results indicate that decreasing the mist "off" time can in

some instances increase root growth, but the increase observed is less than that obtained by simply increasing the sucrose concentration. If the misting cycle has no effect, then growth should have been the same for equivalent sucrose feed rates. A mist tolerance limit for *A. annua* roots in this system is not surprising given the observations of other researchers in terms of root responses to altered misting cycles.

Effects of sugar concentration and composition in shake flasks

Since earlier work suggested that roots in the mist reactor needed a higher concentration of sugar to achieve high growth rates (Fig. 3.3), roots were grown at high initial sucrose concentrations to determine the concentration that produced the highest biomass. Roots grown in shake flasks with sucrose concentrations ranging from 1.8% to 10% (w/v) showed increased biomass up to 6%, after which a decrease was observed (Fig. 3.7). This decrease appears to be an osmotic effect, since roots grown in 5% sucrose grew better than roots grown in either 10% sucrose or an osmotically equal solution of 5% sucrose plus mannitol (Fig. 3.8). Roots grown on mannitol alone showed no growth. Considering that mist reactor experiments all used sucrose at concentrations of 5% or less, the growth decrease of roots in the mist reactor is not osmotically induced.

When the media of roots grown in shake flasks containing 1.8-10% sucrose were analyzed for residual sugar, an interesting trend was noticed (Fig. 3.9). Regardless of the initial sucrose concentration, as long as there was still some sucrose present, the ratio of residual glucose to fructose was relatively constant at 0.85. The same glucose/fructose ratio was consistently observed when residual sugar data from other experiments with *A. annua* roots were examined, including media from roots grown in shake flasks, bubble column reactors, mist reactors (Kim, 2001), and the differential mist reactor described

herein. The physiological significance of this glucose/fructose ratio is unknown. While the roots have an apparent proclivity for this ratio, they do not grow well if it is artificially provided at inoculation (Fig. 3.10). Although the total amount of carbon in each case was equivalent to that in 3% sucrose, roots grown on glucose or glucose/fructose at a 0.85 ratio grew less than half as much as roots grown on sucrose or fructose, which were statistically indistinguishable from each other.

A. annua preferentially consumes glucose before fructose (Kim et al., 2003). Additionally, production of the secondary metabolite, artemisinin, was higher for roots grown on fructose or glucose; 1.6- and 2.9-fold higher, respectively, than roots grown on sucrose (Weathers et al., 2004). It is clear that sugar composition can have a dramatic effect on both biomass production and secondary metabolite accumulation in *A. annua* roots (Weathers et al., 2004), and in other species as well, including *Catharanthus roseus* (Jung et al., 1992), *Vitis vinifera* (Vitrac et al., 2000), and *Atropa belladonna* (Rothe and Drager, 2002). Secondary metabolite production was not the main focus of this research, so mist reactor studies using different sugars other than sucrose were not pursued. However, since it has been shown that *A. annua* roots produce three times more artemisinin in the mist reactor than in liquid systems (Kim et al., 2001), a logical next step would be to attempt to enhance artemisinin production further by incorporating glucose as a carbon source after first growing the roots on sucrose.

Growth decreases although major nutrients are not limiting

When growth of a culture declines, there are mainly two possible but non-exclusive reasons: depletion of key nutrients, and/or the presence of growth inhibitors. Although the growth kinetics of roots grown in the differential mist reactor with 3% or 5% sucrose

(Fig. 3.3) show that the highest growth rate occurs by day 4 for both sugar levels, carbon was not depleted from the medium in this brief time period. Residual sugar data are shown in Fig. 3.4. Analysis of shake flask media indicated that 34-37% of the phosphate, 13-18% of the nitrate, and 5-9% of the total sugar were depleted after 6 days (data not shown). Since a similar trend in phosphate consumption was seen in mist reactor culture media (more than half of the initial phosphate remained (data not shown)), it was likewise assumed that nitrate was not limiting. While Kim (2001) did not assay phosphate levels in mist reactor media, there was no appreciable decrease in nitrate observed until at least 14 days of mist-mode culture had elapsed. The reasons for the slower growth at this early stage (4-6 days) in the differential mist reactor are not known. It remains to be seen if the growth deceleration can be delayed by supplying a higher concentration of sucrose.

Growth inhibitors are not apparent

Hairy roots produce ethylene, in some instances in greater quantities than in untransformed roots (Biondi et al., 1997). Further, growth of *A. annua* roots is significantly decreased by ethylene (Weathers et al., 2005). Ethylene presence in the mist reactor system was assayed using the triple response of pea seedlings (Neljubow, 1901). When the vent gas was fed into a container of pea seedlings, the triple response indicative of ethylene was not evident. This was not an unexpected result since gases are purged every 15 minutes during the misting cycle. At an air flow rate of 4 L min^{-1}, the gas within the growth chamber is exchanged at approximately 80 VVM. Although purging a culture too frequently may also inhibit growth by "stripping" beneficial gases such as CO_2 which has been shown to enhance the growth of other species of hairy roots (DiIorio

et al., 1992a), CO_2 has not been shown to affect the growth rate of *A. annua* significantly (Wyslouzil et al., 2000; Kim et al., 2002a).

To determine if there were any effects on growth from other as yet unknown growth inhibitors in the medium, a shake flask experiment was designed. Three sets of cultures were grown for a total of 14, 21, or 28 days. On day 14, the first set had its medium replaced, and the second did not. As shown in Figure 3.11, there is no significant difference between the two conditions until at least 7 days post-medium replacement. When the converse of that experiment was done using 8 day old shake flask media to grow single hairy roots in a mist reactor, there was a significant increase in growth (Wyslouzil et al., 2000). This burst of growth was probably from the presence of auxins in the medium (Weathers et al., 2005). The third set of flasks had its roots removed on day 14 and was re-inoculated with new roots at the same original inoculum level and grown for an additional 14 days. The resulting rate of biomass increase (ΔDW day^{-1}) was about 63% of the control cultures (Table 3.3, rows A and B). Comparison of control cultures and cultures which received fresh medium on day 14 shows that for the 14-28 day time period, the control cultures grew about 53% as well as the cultures which received fresh medium (Table 3.3, rows G and J) . The growth pattern suggests the presence of growth-enhancing "conditioning" factors that are not initially present in the medium. When the medium, but not the roots, was exchanged, the rate of biomass increase for days 21-28 for the control cultures without medium replacement was only a fraction of that of the cultures that received fresh medium (Table 3.3, rows F and I). If the rates of biomass increase for 7-day intervals are examined, the highest rate occurs between days 14-21 (Table 3.3, row E), and this can only be maintained if the medium is

replaced. At some point between 7 and 21 days, the growth-enhancing effects of conditioned medium become less prevalent, and inhibitory factors and nutrient depletion predominate. One may infer from these shake flask experiments that if growth inhibitors were present in the mist reactor media, they did not accumulate in sufficient levels in the shorter time period used in the differential mist reactor studies (4-6 days). This assumes that roots growing in the mist reactor are producing and exuding the same growth inhibitors, in the same quantities, as in shake flasks. This assumption could be substantiated by using conditioned medium from the mist reactor to grow roots in shake flasks and monitoring effects on biomass accumulation versus fresh medium.

Apparent biomass yield of roots grown in shake flasks versus mist reactor

Comparison of the biomass increase of plant tissue grown in radically different environments yields little information regarding the implications on the inner workings of the component cells. Efficiency of growth, expressed as the biomass yield on a substrate, is a more informative indicator of a tissue's metabolic response to its surroundings. Here we have chosen sucrose as the substrate, though the yield may be expressed in terms of any chosen nutrient. Typically, the carbon source is used, and this is logical considering that *A. annua* roots are >40% (w/w) carbon as determined by elemental analysis (Kim et al., 2003). The apparent biomass yield ($Y_{X/S}$) of transformed roots of *A. annua* in shake flasks is approximately 0.34 g DW / g glucose equivalent in 3% sucrose and 0.32 in 5% sucrose (Fig. 3.12). Similarly, Figure 3.13 shows that $Y_{X/S}$ is about 0.32 and decreases for sucrose concentrations of 6% or higher. Roots grown on fructose had a statistically indistinguishable apparent biomass yield versus roots grown on sucrose, while the value for roots grown on glucose was about 60% lower ($Y_{X/S}$ = 0.19). The values shown in

Figs. 3.12 and 3.13 are comparable to those reported by Kim et al. (2003) for *A. annua* roots grown in shake flasks ($Y_{X/S}$ = 0.35) and bubble column reactors ($Y_{X/S}$ = 0.38); and also for *Atropa belladonna* hairy roots grown in a bubble column reactor ($Y_{X/S}$ = 0.35; Kwok and Doran, 1995), and *Catharanthus roseus* hairy roots grown in shake flasks ($Y_{X/S}$ = 0.40; Bhadra and Shanks, 1997). The apparent biomass yields for *A. annua* roots grown in the differential mist reactor, however, were significantly lower (Fig. 3.14): 0.08 in 3% sucrose and 0.18 in 5% sucrose. As discussed below, it is very likely that these values are artificially low because the mass balance of carbon is incomplete. The nature of the differential mist reactor system made it difficult to track all of the medium, and slight errors in the carbon balance greatly affect the apparent biomass yield calculation due to the minimal amount of biomass used in these studies.

Determination of biomass yield requires a carbon mass balance

As a rough estimate of apparent biomass yield in the differential mist reactor, one may assume that any decrease in media volume is only due to evaporation and retention by roots. However, this assumption very likely overestimates the amount of sugar consumed by the roots by not taking into account the volume of media that exits the coalescer and thus vents unconsumed. The data in Fig. 3.4 only show the total amount of sugar remaining in the medium reservoir at harvest, and are not a true indication of consumption. Consequently, the biomass yield is underestimated. While such an analysis on shake flask data produced results consistent with Kim et al. (2003), mist reactor data gave less reasonable results and were less than half of the value determined by Kim et al. (2003). To try to resolve this discrepancy, the reservoir media levels of all reactor runs were monitored approximately on a daily basis, thereby allowing for a

calculation of the rate of volume decrease. The relative proportions of evaporation and vent loss, however, are still unknown and will vary with the ambient temperature and humidity of the culture room.

In order to determine the amount of evaporation, a bed of 2 mm glass balls was used in place of roots since these had previously been shown to behave similarly to a packed root bed in terms of mist deposition (Whipple, 1995). By using inert packing matter, one can assert that any alteration in the medium composition has occurred via non-consumptive mechanisms, allowing measurement of any background effects inherent in the differential mist reactor system itself. Monitoring the conductivity of the medium every 12 hours for several days suggested that, while changes in conductivity were small variable and perhaps approached the accuracy limit of the conductivity meter used, on average approximately 4.5 mL day^{-1} evaporated from reactor A media, and 18.3 mL day^{-1} evaporated from reactor B media. The difference in evaporation rates can be attributed to the two Nafion humidification units; A had more surface area than B. After removing the inline air humidification units, there was at least a five fold increase in the medium conductivity and volume loss per day versus when humidified air was used (data not shown). This indicated that the air was humidified and evaporative effects were lessened during mist reactor operation, but should not be ignored. The volume loss observed was thus due to both evaporation of water and nutrient mist droplets exiting the coalescer.

One must also consider the relative proportion of sugar being consumed by the roots. Let us assume the apparent biomass yield in the differential mist reactor system is equal to or greater than that observed by Kim (2001) for *A. annua* roots grown in shake flasks and bubble column reactors (approximately 0.40). While this value is much higher

than determined earlier (Fig. 3.14; ignoring vent loss and evaporation), we can justify the higher value as follows. A high respiratory quotient is associated with anaerobic respiration, which is less efficient in the metabolism of carbon than aerobic respiration. Bordonaro and Curtis (2000) observed a consistently higher respiratory quotient for *Hyoscyamus muticus* roots grown in a bubble column reactor than in a more aerobic trickle bed reactor. Since the mist reactor is a highly aerobic environment, roots grown in the differential mist reactor should metabolize nutrients very efficiently; at least as well as in shake flasks.

For an apparent biomass yield of 0.40, if 0.5 g DW of roots have been produced (typical for roots grown 6 d on 3% sucrose), 1.25 g glucose equivalents have been consumed. This amount is 39.58 mL, or 3.5% of 1.125 L 3% sucrose; or 23.75 mL, or 2.1% of 1.125 L 5% sucrose. It is clear that small errors involved in estimating volumes and keeping an accurate account of all the media in the system can have a significant effect on subsequent calculations of apparent biomass yield. The following sample sugar balance illustrates this. If:

$$gluc_{eqv}(i) = gluc_{eqv}(f) + gluc_{eqv}(lost) + \frac{\Delta DW}{Y_{X/S}} \qquad [3.9]$$

where $gluc_{eqv}$ = g glucose equivalents; *i, f, lost* = initial, final, and lost to vent; ΔDW = final – initial DW (g); $Y_{X/S} = \Delta DW / \Delta gluc_{eqv}$ (the apparent biomass yield); and

$$[gluc_{eav}(f)] = \frac{[gluc_{eqv}(f)]_{meas} \times V(f)}{V(f) + V(evap)}, \qquad [3.10]$$

where $[gluc_{eqv}(f)]_{\text{meas}}$ = final concentration as measured by HPLC analysis, and $V(evap)$ is the evaporation volume in L; and

$$[gluc_{eqv}(lost)] = \text{average of } [gluc_{eav}(i)] + [gluc_{eqv}(f)], \qquad [3.11]$$

$$gluc_{eqv}(n) = [gluc_{eqv}(n)] \times V(n) \qquad [3.12]$$

where n = *i, f*, or *lost*; $[gluc_{eqv}(n)]$ = concentration in g L^{-1}; and $V(n)$ = volume in L; and

$$V(lost) = V(f) - V(i) - V(evap), \qquad [3.13]$$

then one can alter various components (i.e. volume, sugar concentration) and note the change in $Y_{X/S}$. In this example, data from reactor run #28A are used.

$gluc_{eqv}(i)$ = 35.53 g

$gluc_{eqv}(f)$ = [(32.12 g L^{-1} × 0.845 L) / (0.845 L + 0.027 L)] × 0.845 L = 26.30 g

$gluc_{eqv}(lost)$ = 31.35 g L^{-1} × 0.280 L = 8.78 g

ΔDW = 0.6928 g

Using these data in Eqn. 3.9 gives an apparent biomass yield $Y_{X/S}$ of 0.54, which is not unreasonable. If we assume the elemental composition of *A. annua* roots is $CH_{1.53}O_{0.66}N_{0.08}$ as estimated by Kim (2001) and that the sugar consumed was converted entirely to biomass, the maximum possible biomass yield is 0.84. It should be noted that the calculated $Y_{X/S}$ values for mist reactor runs using the sugar balance in Eqn. 3.9 showed a range from 0.04 to 0.99, with an overall average of 0.20 for the 34 runs analyzed. The sample calculation above happened to produce the desired result, but it is clear that a satisfactory carbon balance still has not been achieved for the vast majority of the mist reactor data.

Table 3.4 shows the effect of volumetric errors on $Y_{X/S}$ for reactor #28A, specifically errors in measurement of initial volume, evaporation volume, failure to remove all of the liquid from the reactor system, and assuming higher and lower $gluc_{eqv}$

concentrations in the vent loss volume. The largest effect was caused by discrepancies in initial medium volume (and consequently, the initial amount of sugar). Evaporation volume also had an effect, though to a lesser extent. Here we included an error range of ±10% since this volume component is expected to vary with each reactor run, and we were unable to perform a reliable assessment of the evaporation rate. An underestimation of the final volume (by not accounting for liquid left behind in the reactor system or retained by the roots, for example) made little difference, as did the presumed sugar concentration in the vent loss volume. The amount of liquid hold-up in the roots at harvest was assumed to be minimal and was ignored in the previous media volume balance. An examination of unblotted harvested FW and DW data showed that 11 g of liquid media is retained per g DW roots. The equivalent volume of media can be calculated from the density. The DW ranged from 0.7 to 2.3 g; therefore, after subtracting the dry mass, 7.0-23.0 g of liquid media were not taken into account. This is about 6.8-22.3 mL (assuming a sucrose concentration of 3%). Each possible source of error contributes to the accuracy of the overall carbon balance, and none should necessarily be dismissed if there are means to achieve more reliable measurements. Again, because the dry weight differential is not large in this particular system, additive discrepancies in tracking the carbon flow will affect the calculation of apparent biomass yield.

We have so far only viewed the carbon balance in terms of sugar and biomass. The loss of carbon to respiration (as CO_2) in the differential mist reactor may not be negligible. Assuming a growth rate of 0.20 day^{-1} (achieved by *A. annua* roots grown in the mist reactor with 5% sucrose for 4 days), an approximate yield of 0.05 g DW per

mmol CO_2 respired (value between those determined by Kim (2003) for *A. annua* in a bubble column reactor and Ramakrishnan et al. (1999) for *H. muticus* in a trickle bed reactor), and a ΔDW of roots of 0.7 g, then 0.49 g of CO_2 would be respired. Since the initial amount of $gluc_{eqv}$ is 59.21 g, then the amount of carbon respired as CO_2 is only 0.56% of the total initial carbon provided. However, if the apparent biomass yield on sucrose is 0.40, then 1.75 g of $gluc_{eqv}$ are consumed, and the amount of carbon respired is 19% of the total carbon consumed, which is a comparatively significant proportion of the differential we are trying to measure.

Conclusions

The data presented herein support the hypothesis that root inoculum density plays a significant role in the capture of nutrients in a mist reactor as suggested by the aerosol deposition model. Increasing nutrient concentration, particularly sucrose, also increases the growth rate, although the optimum is not yet known. Misting with a high sucrose concentration appears to be essential in order to maintain a high growth rate in the mist reactor. Although altering the frequency of misting should also yield a higher growth rate, this approach is less efficient than increasing the sucrose concentration. Though a decrease in growth rate was observed earlier in the mist reactor than in shake flasks, the cause is still unclear. Major nutrients are not depleted, and the presence of growth inhibitors in the medium needs to be verified experimentally by comparing the effects of conditioned medium from mist reactor-grown roots to that from shake flask-grown roots. Due to the drastic differences in the mode of non-gaseous nutrient delivery in shake flasks versus the mist reactor, the optimum concentration of nutrients necessary for rapid

growth may be very different. While *A. annua* roots grown in shake flasks do not grow well at sucrose concentrations greater than 6% (w/v), it is unknown whether a similar effect will occur in the mist reactor. Roots may actually require a higher nutrient concentration in this environment, as nutrients are captured from small droplets rather than from a continuous liquid milieu. The efficiency of growth, or apparent biomass yield, could not be determined in this system due to the small biomass differential and the inability to trace the carbon flow accurately. Gas-phase reactors show many advantages over liquid-phase reactors, especially in terms of the ability to allow effective gas exchange in densely growing biomass, as indicated here by the successful growth of roots to a high density without additional oxygen supplementation. However, it is clear that the biological responses of transformed roots to this environment are complex, and the potential benefits of this system cannot be realized without further study.

Figure 3.1. Photo of the differential mist reactor, and an example of harvested root mass after 10 d growth (inset). Arrow indicates growth chamber.

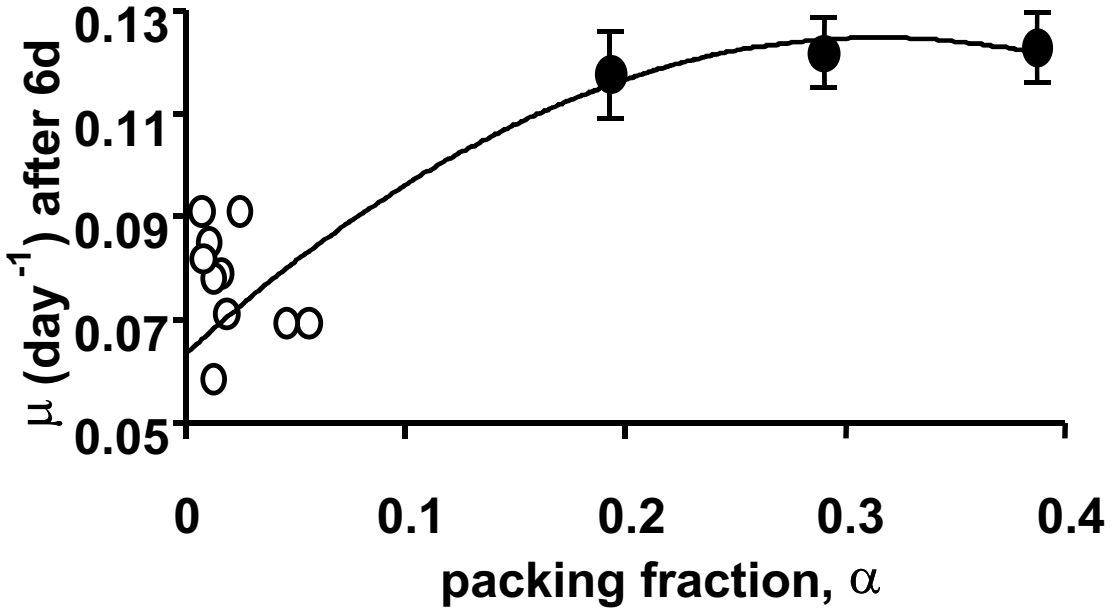

Figure 3.2. Effect of initial packing fraction on the specific growth rate of transformed roots of *A. annua* grown in the differential mist reactor. Roots were inoculated at the indicated packing fraction and grown for 6 days, after which the growth rate was measured from the increase in dry weight. Misting cycle was 15 min on / 15 min off, and air flow during the "on" cycle was 4 L min^{-1}. O = mist reactor data from Kim (2001). Error bars represent one standard deviation.

Table 3.1. Alteration of growth rate (μ) with changes in mist reactor operation and comparative shake flask data

system	packing fraction α	growth rate μ (day^{-1})
bubble column*	0.002-0.006	0.08-0.17
mist reactor*	0.004-0.056	0.06-0.09
mist reactor 3% suc, 6d	0.193-0.387	0.12±0.008
shake flasks 3% suc, 6d	0.008	0.17±0.025
shake flasks 3% suc, 6d	0.290	0.01
mist reactor 5% suc, 4d	0.290	0.20±0.003
shake flasks 5% suc, 4d	0.008	0.19±0.013

*=data from Kim (2001)

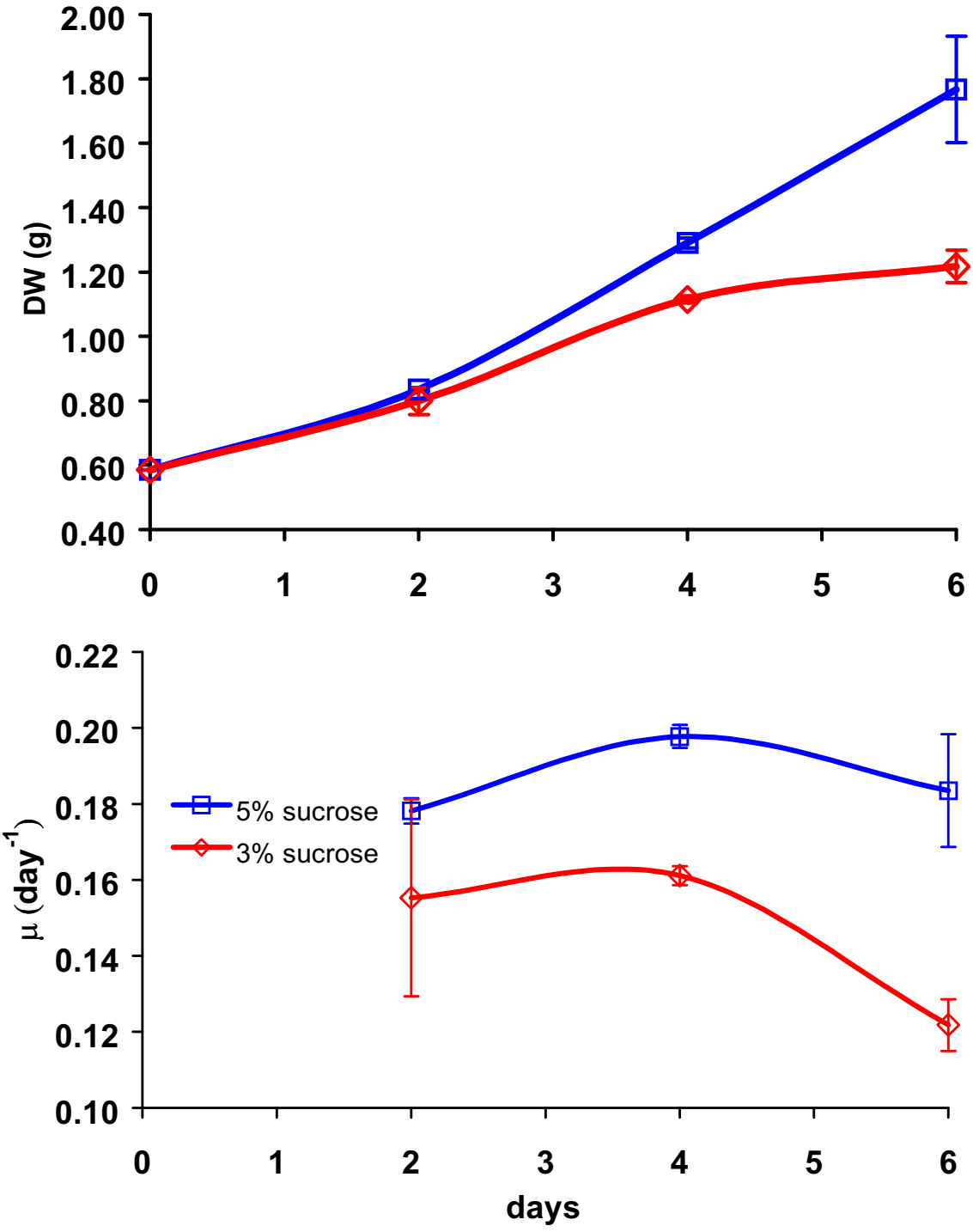

Figure 3.3. Growth of transformed roots of *A. annua* on 3% or 5% sucrose in the differential mist reactor. Top graph, dry weight. Bottom graph, growth rate. Initial packing fraction $\alpha = 0.29$, and other operating conditions were as described in Figure 3.2. Error bars represent one standard deviation.

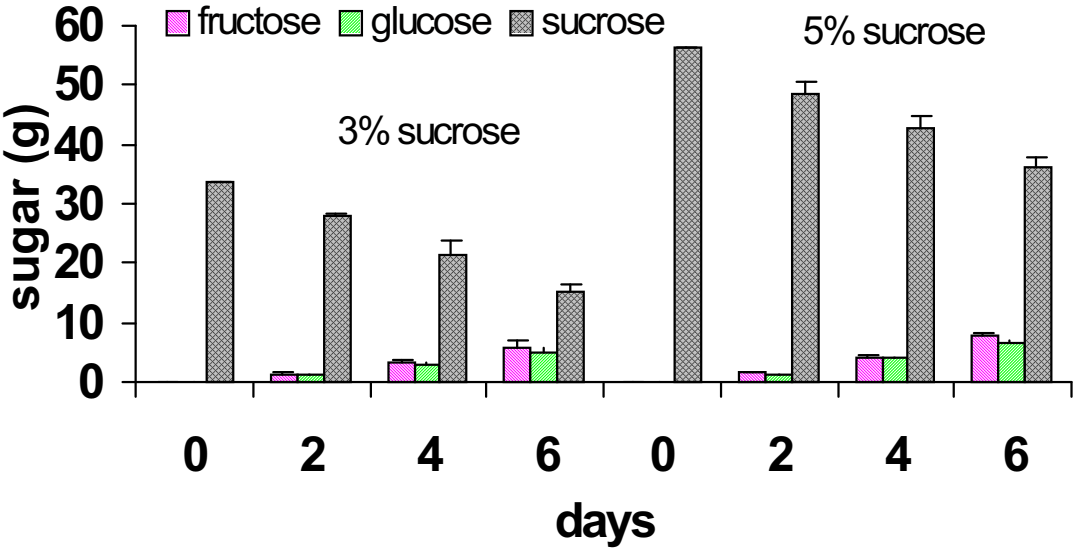

Figure 3.4. Residual sugar content of medium after 0, 2, 4, and 6 days of growth of transformed roots of *A. annua* on 3% or 5% sucrose in the differential mist reactor. Initial packing fraction $\alpha = 0.29$, and other operating conditions were as described in Figure 3.2. Error bars represent one standard deviation.

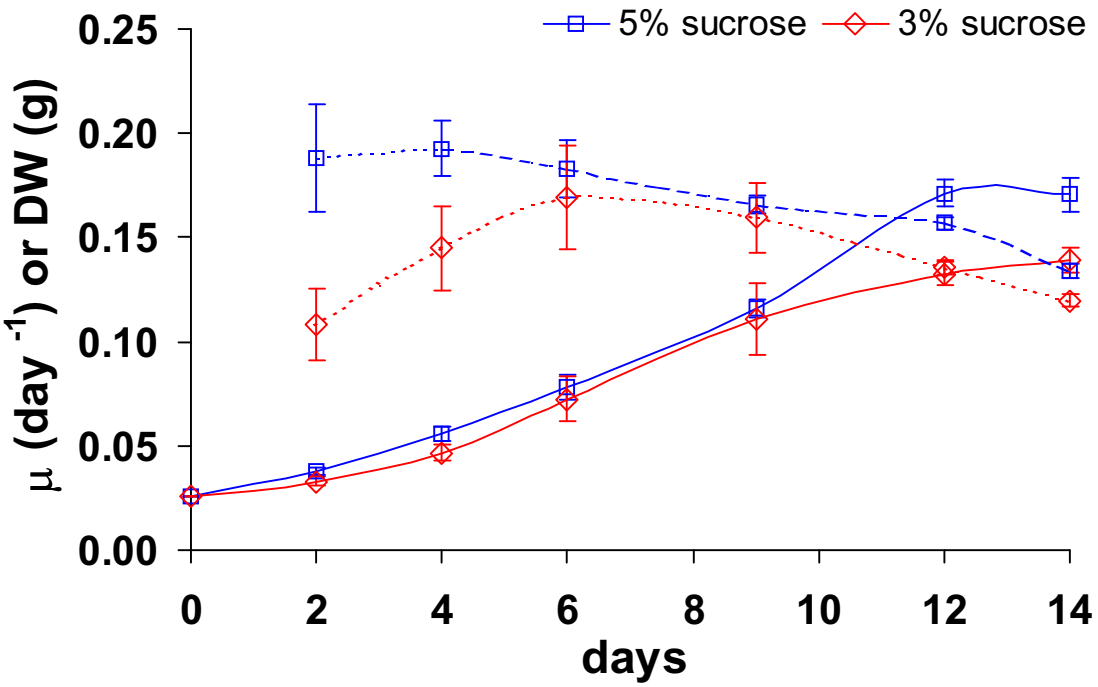

Figure 3.5. Growth of transformed roots of *A. annua* on 3% or 5% sucrose in shake flasks, expressed as DW or growth rate μ. Inoculum was 8 g FW L^{-1} (0.52 g DW L^{-1}) in 50 mL medium. Growth rate, dashed lines; DW, solid lines. Error bars represent one standard deviation.

Table 3.2. Comparison of misting cycles for transformed roots grown in various mist reactors

species	misting cycles (min on/off)	optimum	reference
Beta vulgaris	5/2, 5/6	5/6	DiIorio et al. (1992b)
Carthamus tinctorius	5/5, 5/10, 5/20	5/5	DiIorio et al. (1992b)
Artemisia annua	1/15	n/d	Chatterjee et al. (1997)
A. annua	3/15, 3/30, 3/45, 3/60, 3/90	3/30	Liu et al. (1999)
A. annua	1/15, 5/15, 1/60	1/15	Wyslouzil et al. (2000)
A. annua	5/5, 5/15, 7/15, 8/15, 15/15	n/d	Kim (2001)
A. annua	15/3, 15/15	15/15	This work

n/d = not determined

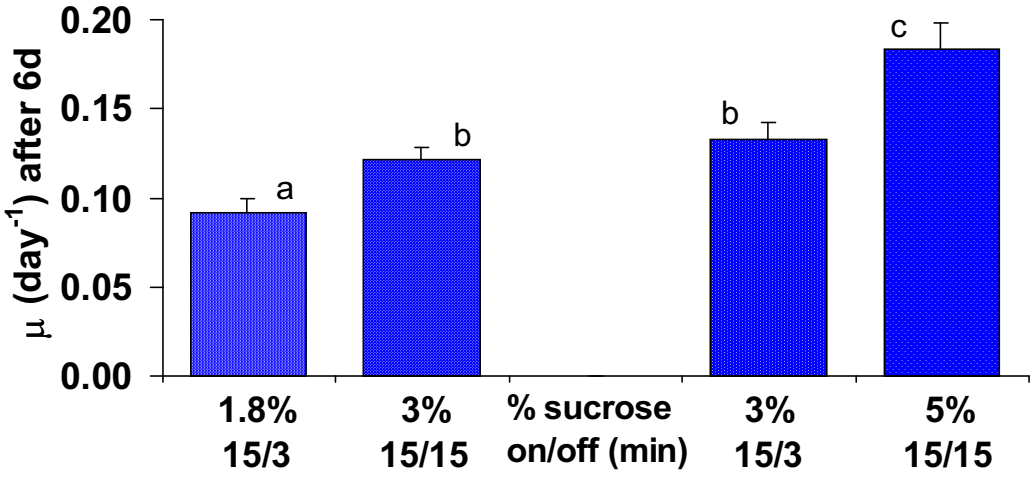

Figure 3.6. Effect of misting cycle on the specific growth rate of transformed roots of *A. annua* in the differential mist reactor. A 1.8% sucrose feed at a misting cycle of 15/3 is equivalent to a 3% sucrose feed at a misting cycle of 15/15; and a 3% sucrose feed at a misting cycle of 15/3 is equivalent to a 5% sucrose feed at a misting cycle of 15/15. Initial packing fraction $\alpha = 0.29$, and other operating conditions were as described in Figure 3.2. Error bars represent one standard deviation. Letters indicate statistical significance at P = 0.05.

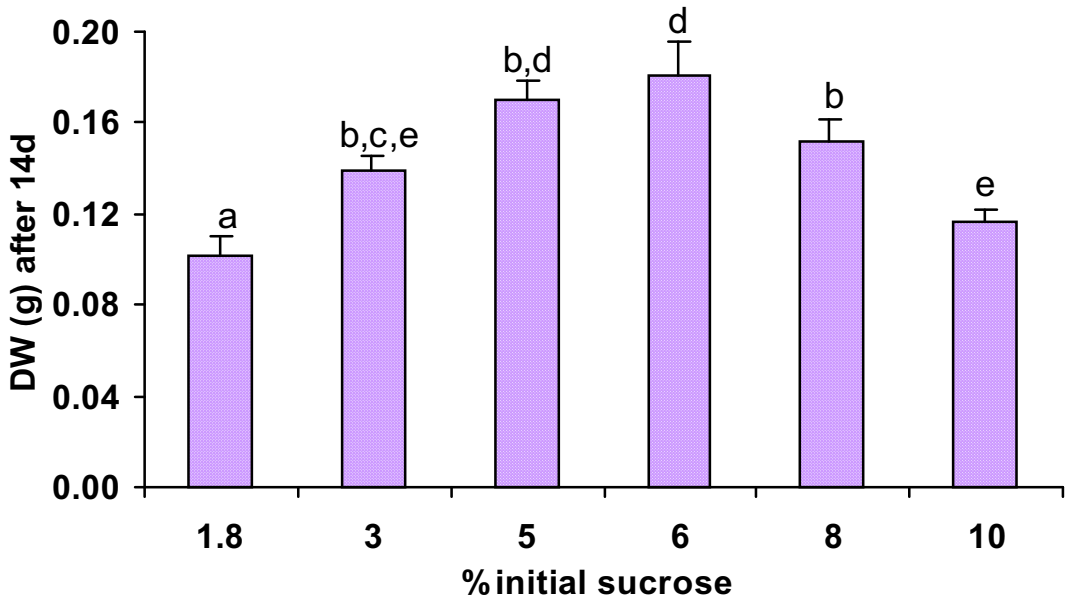

Figure 3.7. Effect of initial sucrose concentration on growth of transformed roots of *A. annua* in shake flasks. Initial inoculum was 8 g FW L^{-1} (0.52 g DW L^{-1}) in 50 mL B5 medium with the indicated concentration of sucrose. Error bars represent one standard deviation. Letters indicate statistical significance at P = 0.05.

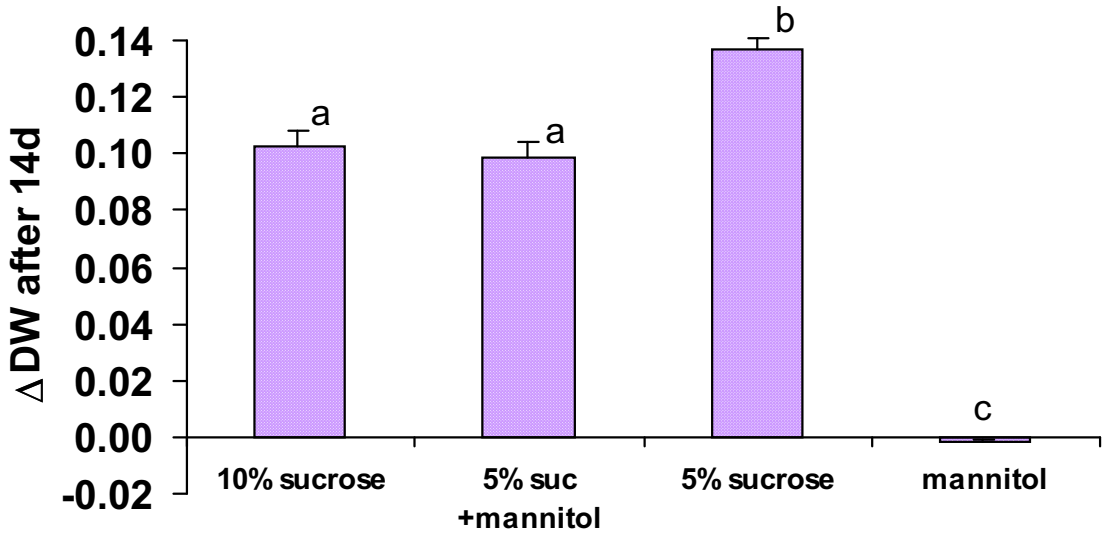

Figure 3.8. Effect of initial sucrose concentration and mannitol on growth (as change in dry weight) of transformed roots of *A. annua* in shake flasks. Initial inoculum was 8 g FW L^{-1} (0.52 g DW L^{-1}) in 50 mL B5 medium with the indicated concentration of sucrose ± mannitol. The solution of 5% sucrose + mannitol was osmotically equivalent to 10% sucrose, and the mannitol control was osmotically equivalent to 5% sucrose. Error bars represent one standard deviation. Letters indicate statistical significance at P = 0.05.

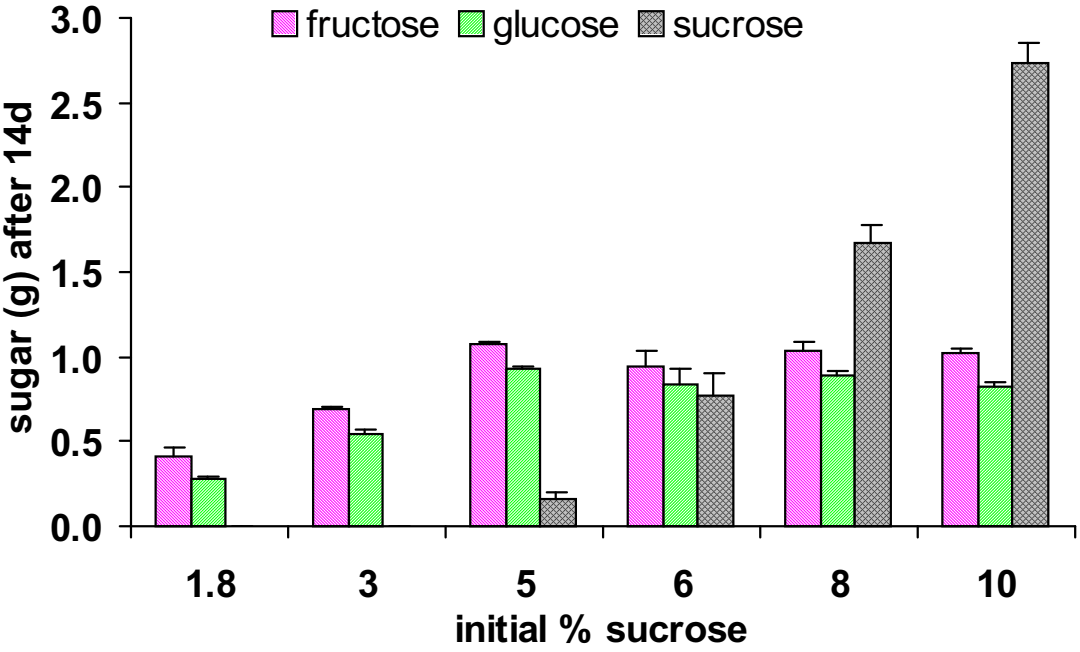

Figure 3.9. Residual sugar content after 14 days of growth of transformed roots of *A. annua* on various sucrose concentrations in shake flasks. Inoculum was 8 g FW L^{-1} (0.52 g DW L^{-1}) in 50 mL B5 medium. Error bars represent one standard deviation.

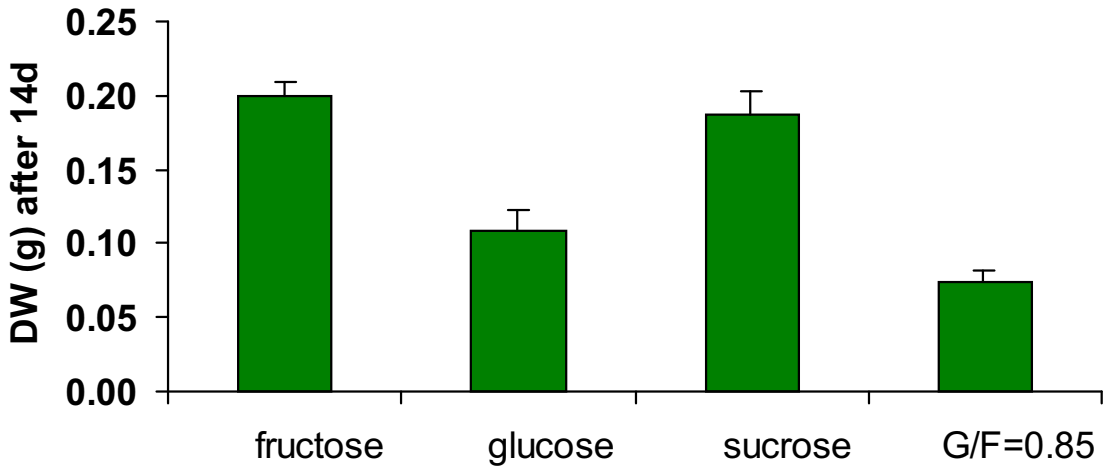

Figure 3.10. Effect of individual sugars and monosaccharide ratio on growth of transformed roots of *A. annua* in shake flasks. Initial inoculum was 10 g FW L^{-1} (0.65 g DW L^{-1}) in 50 mL B5 medium for sucrose, fructose, and glucose (data from Weathers et al., 2004); and 9 g FW L^{-1} (0.585 g DW L^{-1}) for glucose/fructose = 0.85. Carbon amounts in each case are equivalent to that in 3% sucrose. Error bars represent one standard deviation.

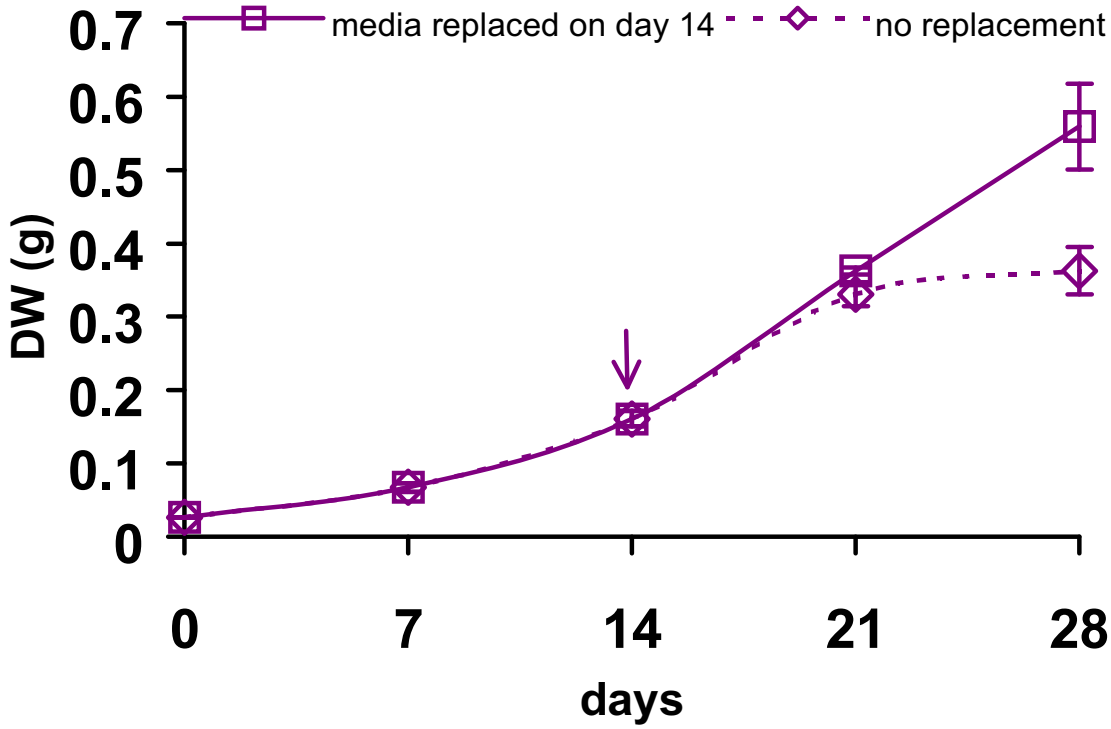

Figure 3.11. Effect of media replacement on growth of transformed roots of *A. annua* in shake flasks. On day 14 (arrow) the media in one set of flasks was drained and replaced with 50 mL of fresh media. Roots were inoculated with equivalent amounts for each experimental condition. Error bars represent one standard deviation.

Table 3.3. Effect of spent medium and medium replacement on growth of transformed roots of *A. annua* in shake flasks.

condition		biomass (g FW)	days (Δ)	ΔDW (mg day^{-1})
A	new roots in fresh medium at day 0	0.45	0-14 (14d)	9.63
B	new roots into spent medium at day 14	0.45	14-28 (14d)	6.06
C	fresh medium at day 0	2.24	0-7 (7d)	5.89
D			7-14 (7d)	13.37
E			14-21 (7d)	24.25
F			21-28 (7d)	6.06
G			14-28 (14d)	15.16
H	fresh medium at day 14	2.24	14-21 (7d)	28.78
I			21-28 (7d)	28.14
J			14-28 (14d)	28.46

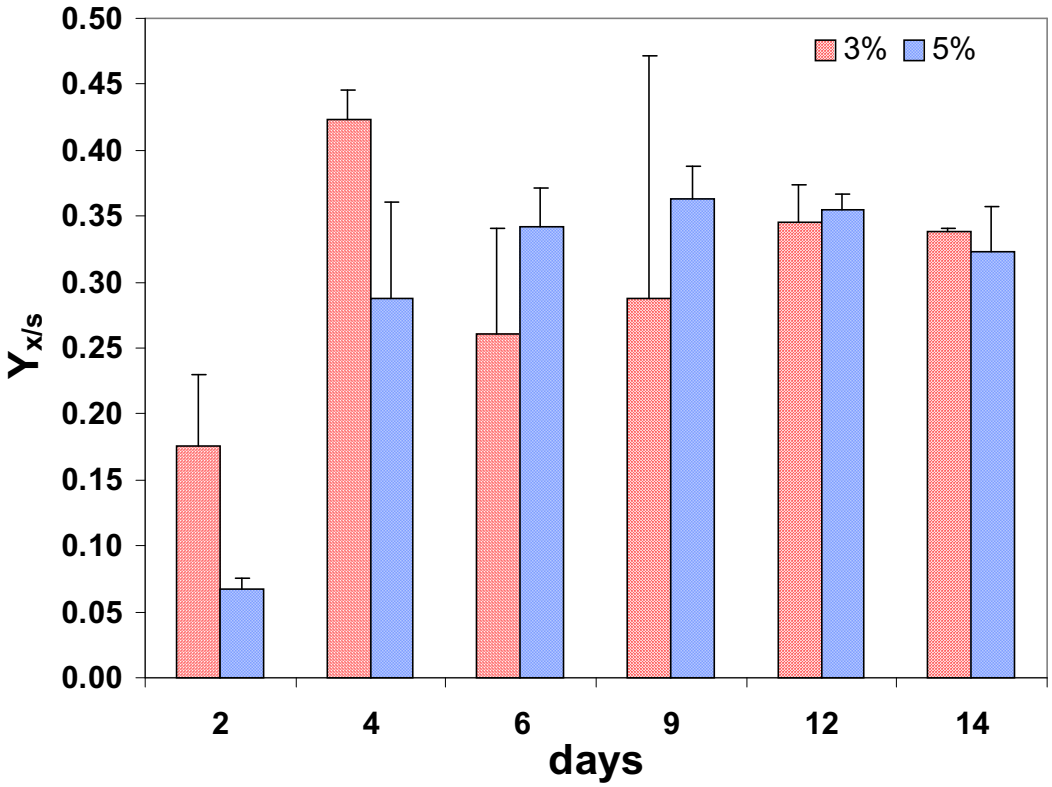

Figure 3.12. Apparent biomass yield ($Y_{X/S}$ = Δ g DW / Δ g glucose equivalent) at different time points for transformed roots of *A. annua* grown on 3% or 5% sucrose in shake flasks for a total of 14 days. Initial inoculum was 8 g FW L^{-1} (0.52 g DW L^{-1}) in 50 mL B5 medium. Error bars represent one standard deviation.

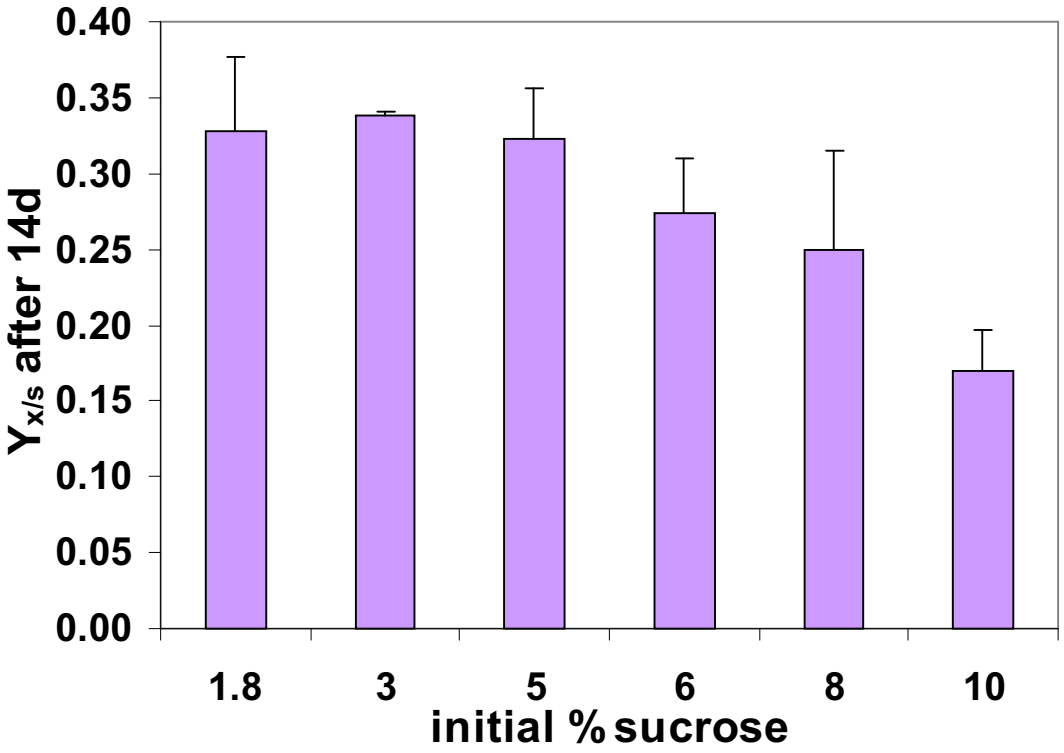

Figure 3.13. Apparent biomass yield ($Y_{X/S}$ = Δ g DW / Δ g glucose equivalent) after 14 days for transformed roots of *A. annua* grown on various initial sucrose concentrations in shake flasks. Initial inoculum was 8 g FW L^{-1} (0.52 g DW L^{-1}) in 50 mL B5 medium. Error bars represent one standard deviation.

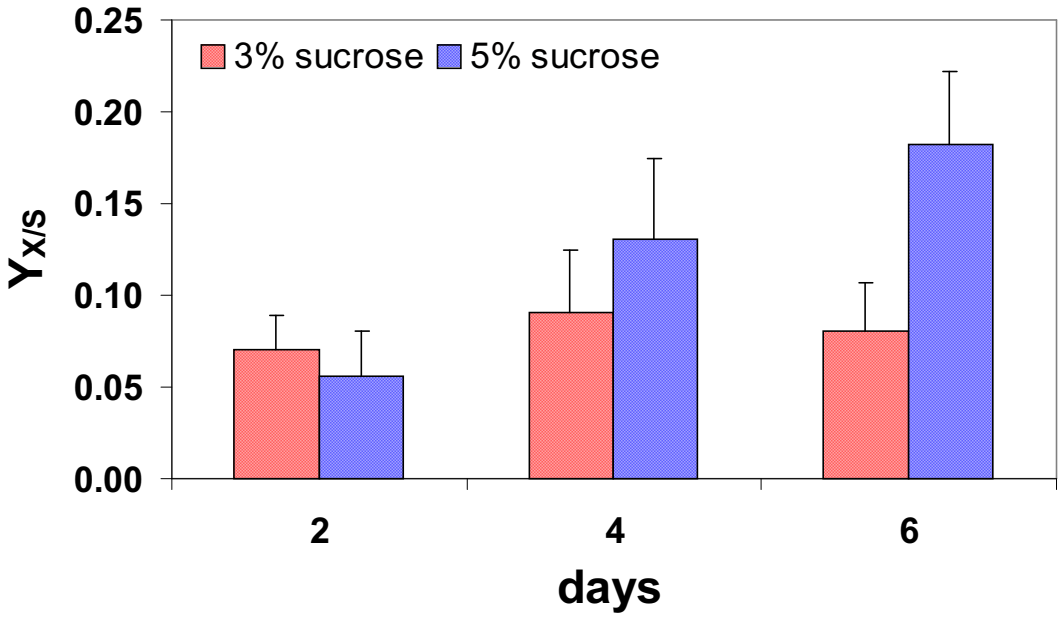

Figure 3.14. Apparent biomass yield ($Y_{X/S}$ = Δ g DW / Δ g glucose equivalent) after 2, 4, and 6 days of growth of transformed roots of *A. annua* on 3% or 5% sucrose in the differential mist reactor, not taking into account evaporation or media loss from the vent. Initial packing fraction $\alpha = 0.29$, misting cycle was 15 min on / 15 min off, and air flow during the "on" cycle was 4 L min^{-1}. Error bars represent one standard deviation.

Table 3.4. Effect of volumetric errors and estimation of sugar concentration on calculation of apparent biomass yield of roots grown in reactor run #28A.

condition	apparent biomass yield, $Y_{X/S}$ (g DW / g $gluc_{eqv}$); (% change)
original calculation	0.54
-1% error in $V(i)$	0.74 (+37%)
+1% error in $V(i)$	0.42 (-22%)
-10% error in $V(evap)$	0.62 (+15%)
+10% error in $V(evap)$	0.47 (-13%)
10, 20, or 30 mL left in reactor system	0.53 (-2%)
$[gluc_{eqv}(lost)] = [gluc_{eqv}(i)]$	0.56 (+4%)
$[gluc_{eqv}(lost)] = [gluc_{eqv}(f)]$	0.51 (-6%)

4

Overall conclusions and future work

The sensitivity of plant tissues to small changes in the gas-phase composition of their culture environment implies a need for the ability to control or alter it with relative ease. Additionally, since volumetric productivity is contingent upon the density to which one can grow valuable biomass, the capacity to deliver gases to highly packed tissues becomes an important issue. Gas transport is not a trivial matter in a liquid-phase culture system due to low gas solubility and shear sensitivity of plant tissues to the stirring, bubbling, or sparging necessary to enhance mass transfer. Consequently, it is advantageous to use gas-phase bioreactors when dense tissue and/or gas manipulation are desired or required. Previous work has shown that plant tissue (specifically, transformed roots) grown in a mist reactor respond to this environment in complex ways. The work presented here has attempted to answer some essential questions about the behavior of roots in a gas-phase mist reactor in order to develop better ways of designing and exploiting the system to maximize its benefits for this application.

Earlier work used a hybrid reactor wherein the roots were grown in liquid phase until they became immobilized, and then the growth chamber was drained of medium and run as a gas-phase (mist) reactor (Kim, 2001). In order to decrease the length of time the roots were exposed to the liquid-phase environment so that a more accurate appraisal of tissue response to the gas-phase environment could be made, a method of rapid attachment was sought. Roots of three different species (*Artemisia annua*, *Beta vulgaris*,

and *Arabidopsis thaliana*) adhered to a poly-L-lysine coated polypropylene substrate within minutes, although the mechanism of attachment was not completely understood (Towler and Weathers, 2003). This work was not pursued extensively since shortly thereafter an interesting hypothesis regarding the effect of root inoculum density on growth seemed worthy of immediate investigation.

The mist reactor was altered by greatly reducing the size of the growth chamber so that it could be manually packed with roots, thereby eliminating the liquid-phase mode completely. It was demonstrated that by using higher initial inoculum densities than those studied by Kim (2001), growth could be increased. This supported the hypothesis that the capture of nutrients from the mist was highly dependent on the density of the biomass, as suggested by the aerosol deposition model (Wyslouzil et al., 1997). Similarly, increasing nutrient concentration, particularly sucrose, also increased the growth rate, although the optimum has not yet been determined. Additional studies are recommended. Misting with a high sucrose concentration may be essential to maintaining a high growth rate in the mist reactor. Though a growth rate decrease was observed after only 4-6 days in the mist reactor, the cause is not certain. Media analysis showed that major nutrients were not exhausted in this time period. Gaseous growth inhibitors, specifically ethylene, were also not detected. While it is unlikely that growth inhibitors in the medium had accumulated sufficiently to affect growth, this needs to be verified experimentally by comparing the effects of conditioned medium from mist reactor-grown roots to that from shake flask-grown roots. Although altering the frequency of misting should have also yielded higher growth rates according to the mist deposition model, this approach was not as successful as increasing the sucrose concentration. The optimum concentration of

nutrients that are required for rapid growth in the mist reactor may vary from that observed in shake flask culture because of the differences in the mode of nutrient delivery. *A. annua* roots in shake flasks do not grow well at sucrose concentrations greater than 6% (w/v), but since the nutrients are captured from small droplets rather than from a continuous liquid phase in the mist reactor, roots may require a higher nutrient concentration in this environment.

The small biomass differential and the inability to track the carbon flow completely made it impossible to calculate the apparent biomass yield in the differential mist reactor. If the volume of media was decreased such that more exact measurements of changes in concentration and volume could be made, perhaps the biomass yield could be determined. However, altering the biomass/medium ratio, which was kept constant in these studies, may introduce additional effects.

The overall results of these studies showed great promise, in that we were able to improve the growth rate in the mist reactor such that it paralleled that observed in shake flasks, which not only supported our proposed hypotheses but also indicated that a mist-based nutrient delivery system is worthy of further research.

5

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Appendix A. Raw data

Mist reactor experiments

Shake flask experiments

Reactor #	0
Date	July 5, 2001
Inoculum and age	3.0 g FW T16, 15d
Culture chamber	heat-sealed Reynold's oven bag (horizontal)
Medium	filtered B5, 3% sucrose
Air flow	5 L/min, room air
Misting cycle	1 min on / 3 min off
Culture time	12.0 days

Comments: horizontal bag; misting problems; cloudy day 12

Initial:		Final:	
FW (g)	3.0	FW	7.81
DW (g)	0.4582	DW	n/d
volume (L)	1.5	volume	n/d
packing fraction	0.033	packing fraction	0.078

****

Reactor #	1A
Date	August 12, 2001
Inoculum and age	6.0 g FW T16, 14d
Culture chamber	heat-sealed Reynold's oven bag
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, room air
Misting cycle	5 min on / 15 min off
Culture time	4.0 days

Comments: dry roots day 4 (hole in tubing to mist generator)

Initial:		Final:	
FW (g)	6.0	FW	n/d
DW (g)	0.390	DW	n/d
volume (L)	1.5	volume	n/d
packing fraction	0.177	packing fraction	n/d

****

Reactor #	1B
Date	August 3, 2001
Inoculum and age	6.0 g FW T16, 15d
Culture chamber	heat-sealed Reynold's oven bag
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, room air
Misting cycle	5 min on / 15 min off
Culture time	6.0 days

Comments: cloudy day 6 (reactor leak)

Initial:		Final:	
FW (g)	6.0	FW	8.54
DW (g)	0.390	DW	0.5863
volume (L)	1.5	volume	n/d
packing fraction	0.177	packing fraction	0.252

Reactor #	2A
Date	August 11, 2001
Inoculum and age	6.0 g FW T16, 16d
Culture chamber	heat-sealed Reynold's oven bag
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, room air
Misting cycle	5 min on / 15 min off
Culture time	9.0 days

Comments: cloudy day 9; possible misting problems, low airflow

Initial:		Final:	
FW (g)	6.0	FW	13.83
DW (g)	0.390	DW	n/d
volume (L)	1.5	volume	n/d
packing fraction	0.177	packing fraction	0.407

****

Reactor #	2B
Date	August 11, 2001
Inoculum and age	6.0 g FW T16, 16d
Culture chamber	heat-sealed Reynold's oven bag
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, room air
Misting cycle	5 min on / 15 min off
Culture time	0 days

Comments: culture bag broke after reactor initiation

Initial:	
FW (g)	6.0
DW (g)	0.390
volume (L)	1.5
packing fraction	0.177

****

Reactor #	2.5B
Date	August 17, 2001
Inoculum and age	6.0 g FW T16, 15d
Culture chamber	heat-sealed Reynold's oven bag
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, room air
Misting cycle	5 min on / 15 min off
Culture time	3 days

Comments: big media leak; media gone day 3

Initial:	
FW (g)	6.0
DW (g)	0.390
volume (L)	1.5
packing fraction	0.177

Reactor #	3A
Date	August 24, 2001
Inoculum and age	6.0 g FW T16, 16d
Culture chamber	heat-sealed Reynold's oven bag
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, room air
Misting cycle	5 min on / 15 min off
Culture time	6 days

Comments: cloudy day 6

Initial:		Final:	
FW (g)	6.0	FW	7.47
DW (g)	0.390	DW	0.5278
volume (L)	1.5	volume	n/d
packing fraction	0.177	packing fraction	0.220

****

Reactor #	3B
Date	August 24, 2001
Inoculum and age	6.0 g FW T16, 16d
Culture chamber	heat-sealed Reynold's oven bag
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, room air
Misting cycle	5 min on / 15 min off
Culture time	3 days

Comments: leaked; media gone day 3

Initial:	
FW (g)	6.0
DW (g)	0.390
volume (L)	1.5
packing fraction	0.177

****

Reactor #	4A
Date	September 14, 2001
Inoculum and age	6.0 g FW T16, 15d
Culture chamber	heat-sealed Reynold's oven bag
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, room air
Misting cycle	5 min on / 15 min off
Culture time	6 days

Comments: cloudy day 6 (leaky bag)

Initial:		Final:	
FW (g)	6.0	FW	9.41
DW (g)	0.390	DW	0.7823
volume (L)	1.5	volume	n/d
packing fraction	0.177	packing fraction	0.277

Reactor #	4B
Date	September 15, 2001
Inoculum and age	6.0 g FW T16, 16d
Culture chamber	heat-sealed Reynold's oven bag
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, room air
Misting cycle	5 min on / 15 min off
Culture time	6 days

Comments: cloudy day 5 (leaky bag)

Initial:		Final:	
FW (g)	6.0	FW	7.54
DW (g)	0.390	DW	0.6202
volume (L)	1.5	volume	n/d
packing fraction	0.177	packing fraction	0.222

****

Reactor #	5A
Date	October 26, 2001
Inoculum and age	6.0 g FW T16, 15d
Culture chamber	heat-sealed biohazard bag (VWR cat#11215-825)
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, room air
Misting cycle	5 min on / 15 min off
Culture time	1 day

Comments: cloudy day 1; found leak at top of mist generator

Initial:	
FW (g)	6.0
DW (g)	0.390
volume (L)	1.5

****

Reactor #	5B
Date	October 26, 2001
Inoculum and age	6.0 g FW T16, 15d
Culture chamber	heat-sealed biohazard bag (VWR cat#11215-825)
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, room air
Misting cycle	5 min on / 15 min off
Culture time	3 days

Comments: cloudy day 2; media is leaking into humidifier unit

Initial:		Final:	
FW (g)	6.0	FW	6.66
DW (g)	0.390	DW	0.5017
volume (L)	1.5	volume	n/d
packing fraction	0.177	packing fraction	0.196

Reactor #	6A
Date	November 30, 2001
Inoculum and age	6.0 g FW T16, 15d
Culture chamber	heat-sealed biohazard bag (VWR cat#11215-825)
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, room air
Misting cycle	15 min on / 15 min off
Culture time	6 days

Comments: cloudy day 6

Initial:		Final:	
FW (g)	6.0	FW	6.98
DW (g)	0.390	DW	0.6018
volume (L)	1.5	volume	n/d
packing fraction	0.190	packing fraction	0.220

****

Reactor #	6B1+2
Date	December 1, 2001
Inoculum and age	6.0 g FW T16, 16d
Culture chamber	heat-sealed biohazard bag (VWR cat#11215-825)
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, room air
Misting cycle	15 min on / 15 min off
Culture time	2 days

Comments: 1: melted fitting; 2: media gone day 2 (mist generator leak)

Initial:	
FW (g)	6.0
DW (g)	0.390
volume (L)	1.5
packing fraction	0.177

Reactor #	7A
Date	December 21, 2001
Inoculum and age	6.0 g FW T16, 15d
Culture chamber	heat-sealed biohazard bag (VWR cat#11215-825)
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, room air
Misting cycle	15 min on / 15 min off
Culture time	14.8 days
Comments: clean	

Initial:		Final:	
FW (g)	6.0	FW	17.08
DW (g)	0.390	DW	1.7215
volume (L)	1.5	volume	n/d
conductivity (mS/cm)	n/d	conductivity	2380
packing fraction	0.190	packing fraction	0.503

sugar concentration (g/L)	fructose	glucose	sucrose	glucose equivalents
initial	0	0	30	31.5789
final	6.7606	5.1082	17.3974	30.1800

phosphate (g/L)	
initial	0.1033
final	0.0740

Reactor #	7B
Date	December 21, 2001
Inoculum and age	none
Culture chamber	heat-sealed biohazard bag (VWR cat#11215-825)
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, room air
Misting cycle	15 min on / 15 min off
Culture time	0 days

Comments: equipment failure (autoclave damaged reactor)

****

Reactor #	8A		
Date	January 5, 2002		
Inoculum and age	6.0 g FW T16, 16d		
Culture chamber	heat-sealed biohazard bag (VWR cat#11215-825)		
Medium	filtered B5, 3% sucrose		
Air flow	4 L/min, room air		
Misting cycle	15 min on / 15 min off		
Culture time	6 days		

Comments: drips; glued; still looks clean

Initial:		Final:	
FW (g)	6.0	FW	8.49
DW (g)	0.390	DW	0.8066
volume (L)	1.5	volume	n/d
conductivity (mS/cm)	n/d	conductivity	2580
packing fraction	0.190	packing fraction	0.250

sugar concentration (g/L)	fructose	glucose	sucrose	glucose equivalents
initial	0	0	30	31.5789
final	0	0	29.5686	31.1248

phosphate (g/L)	
initial	0.1033
final	0.1031

****

Reactor #	8B
Date	January 4, 2002
Inoculum and age	6.0 g FW T16, 15d
Culture chamber	heat-sealed biohazard bag (VWR cat#11215-825)
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, room air
Misting cycle	15 min on / 15 min off
Culture time	1 day

Comments: bag split day 1

Initial:	
FW (g)	6.0
DW (g)	0.390
volume (L)	1.5
packing fraction	0.177

Reactor #	9B
Date	January 12, 2002
Inoculum and age	6.0 g FW T16, 16d
Culture chamber	heat-sealed biohazard bag (VWR cat#11215825)
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, room air
Misting cycle	15 min on / 15 min off
Culture time	5 days

Comments: cloudy day 5; leaky bag and mist generator

Initial:	
FW (g)	6.0
DW (g)	0.390
volume (L)	1.5
packing fraction	0.157

****

Reactor #	10A
Date	January 25, 2002
Inoculum and age	6.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, room air
Misting cycle	15 min on / 15 min off
Culture time	6 days

Comments: cloudy day 6

Initial:		Final:	
FW (g)	6.0	FW	10.28
DW (g)	0.390	DW	0.9088
volume (L)	1.5	volume	n/d
packing fraction	0.193	packing fraction	0.307

Reactor #	11A
Date	February 8, 2002
Inoculum and age	6.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, room air
Misting cycle	15 min on / 15 min off
Culture time	14.8 days

Comments: clean; lower mist due to slime under mist generator

Initial:		Final:	
FW (g)	6.0	FW	13.91
DW (g)	0.390	DW	1.3409
volume (L)	1.5	volume	1.345
conductivity (mS/cm)	n/d	conductivity	2920
packing fraction	0.231	packing fraction	0.488

sugar concentration (g/L)	fructose	glucose	sucrose	glucose equivalents
initial	0	0	30	31.5789
final	3.1055	2.1417	21.3608	27.7322

phosphate (g/L)	
initial	0.1033
final	0.0814

****

Reactor #	11B
Date	February 8, 2002
Inoculum and age	6.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, room air
Misting cycle	15 min on / 15 min off
Culture time	14.8 days

Comments: clean; misting problems (bubble under generator)

Initial:		Final:	
FW (g)	6.0	FW	17.65
DW (g)	0.390	DW	1.7897
volume (L)	1.5	volume	1.115
conductivity (mS/cm)	n/d	conductivity	2380
packing fraction	0.207	packing fraction	0.569

sugar concentration (g/L)	fructose	glucose	sucrose	glucose equivalents
initial	0	0	30	31.5789
final	5.8861	4.4863	18.4345	29.7771

phosphate (g/L)	
initial	0.1033
final	0.0786

Reactor #	12A
Date	March 1, 2002
Inoculum and age	6.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, room air
Misting cycle	15 min on / 10 min off
Culture time	14.8 days

Comments: clean

Initial:		Final:	
FW (g)	6.0	FW	16.95
DW (g)	0.390	DW	1.6789
volume (L)	0.75	volume	0.510
conductivity (mS/cm)	n/d	conductivity	2580
packing fraction	0.193	packing fraction	0.570

sugar concentration(g/L)	fructose	glucose	sucrose	glucose equivalents
initial	0	0	30	31.5789
final	6.5753	4.7097	13.1452	25.1221

phosphate (g/L)	
initial	0.1033
final	0.0544

Reactor #	12B
Date	March 1, 2002
Inoculum and age	6.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, room air
Misting cycle	15 min on / 10 min off
Culture time	7 days

Comments: cloudy day 7

Initial:		Final:	
FW (g)	6.0	FW	n/d
DW (g)	0.390	DW	n/d
volume (L)	0.750	volume	n/d
packing fraction	0.193	packing fraction	n/d

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Reactor #	13B
Date	March 16, 2002
Inoculum and age	6.0 g FW T16, 16d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, room air
Misting cycle	15 min on / 15 min off
Culture time	9.1 days

Comments: cloudy day 8

Initial:		Final:	
FW (g)	6.0	FW	8.32
DW (g)	0.390	DW	0.7222
volume (L)	0.750	volume	n/d
packing fraction	0.211	packing fraction	0.268

****

Reactor #	13A
Date	March 23, 2002
Inoculum and age	6.0 g FW T16, 16d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, room air
Misting cycle	15 min on / 15 min off
Culture time	6 days

Comments: cloudy day 4

Initial:		Final:	
FW (g)	6.0	FW	9.85
DW (g)	0.390	DW	0.9842
volume (L)	0.750	volume	n/d
conductivity (mS/cm)	n/d	conductivity	4040
packing fraction	0.193	packing fraction	0.318

Reactor # 14B
Date March 29, 2002
Inoculum and age 6.0 g FW T16, 15d
Culture chamber 50 mL centrifuge tube
Medium filtered B5, 3% sucrose
Air flow 4 L/min, room air
Misting cycle 15 min on / 15 min off
Culture time 3 days
Comments: cloudy day 3

Initial:		Final:	
FW (g)	6.0	FW	6.31
DW (g)	0.390	DW	0.5531
volume (L)	0.750	volume	n/d
conductivity (mS/cm)	3550	conductivity	3790
packing fraction	0.193	packing fraction	0.204

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Reactor # 15A
Date April 13, 2002
Inoculum and age 6.0 g FW T16, 16d
Culture chamber 50 mL centrifuge tube
Medium filtered B5, 3% sucrose
Air flow 4 L/min, room air
Misting cycle 15 min on / 15 min off
Culture time 3.05 days
Comments: leaks, tubing kinks, misting problems

Initial:		Final:	
FW (g)	6.0	FW	4.98
DW (g)	0.390	DW	0.4365
volume (L)	0.750	volume	n/d
packing fraction	0.179	packing fraction	n/d

****

Reactor # 15B
Date April 13, 2002
Inoculum and age 6.0 g FW T16, 16d
Culture chamber 50 mL centrifuge tube
Medium filtered B5, 3% sucrose
Air flow 4 L/min, room air
Misting cycle 15 min on / 15 min off
Culture time 0 days
Comments: broke media reservoir during setup

Initial:		Final:	
FW (g)	6.0	FW	n/d
DW (g)	0.390	DW	n/d
volume (L)	0.750	volume	n/d

Reactor #	16A
Date	April 18, 2002
Inoculum and age	6.0 g FW T16, 14d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, room air
Misting cycle	15 min on / 15 min off
Culture time	10.25 days

Comments: clean

Initial:		Final:	
FW (g)	6.0	FW	9.54
DW (g)	0.390	DW	0.8417
volume (L)	0.750	volume	0.646
conductivity (mS/cm)	3970	conductivity	3790
packing fraction	0.193	packing fraction	0.285

sugar concentration (g/L)	fructose	glucose	sucrose	glucose equivalents
initial	0	0	30	31.5789
final	4.1774	3.0938	22.1762	30.6146

phosphate (g/L)	
initial	0.1033
final	0.0843

****

Reactor #	16B
Date	April 18,2002
Inoculum and age	6.0 g FW T16, 14d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, room air
Misting cycle	15 min on / 15 min off
Culture time	10.25 days

Comments: clean; low air flow (~2.75 L/min) day 810

Initial:		Final:	
FW (g)	6.0	FW	15.02
DW (g)	0.390	DW	1.4112
volume (L)	0.750	volume	0.646
conductivity (mS/cm)	3970	conductivity	3720
packing fraction	0.193	packing fraction	0.448

sugar concentration (g/L)	fructose	glucose	sucrose	glucose equivalents
initial	0	0	30	31.5789
final	15.7837	12.8316	6.5138	35.4719

phosphate (g/L)	
initial	0.1033
final	0.0533

Reactor #	17B1
Date	May 3, 2002
Inoculum and age	6.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, room air
Misting cycle	15 min on / 15 min off
Culture time	2.875 days

Comments: cloudy day 3 (leaks)

Initial:		Final:	
FW (g)	6.0	FW	5.83
DW (g)	0.390	DW	n/d
volume (L)	0.750	volume	0.595
conductivity (mS/cm)	3990	conductivity	4190
packing fraction	0.193	packing fraction	n/d

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Reactor #	17B2
Date	May 9, 2002
Inoculum and age	6.0 g FW T16, 14d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, room air
Misting cycle	15 min on / 15 min off
Culture time	6 days

Comments: cloudy day 6; misting problems.

Initial:		Final:	
FW (g)	6.0	FW	n/d
DW (g)	0.390	DW	n/d
volume (L)	0.750	volume	n/d
conductivity (mS/cm)	3910	conductivity	n/d
packing fraction	0.193	packing fraction	n/d

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Reactor #	17A
Date	May 3, 2002
Inoculum and age	6.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, room air
Misting cycle	15 min on / 15 min off
Culture time	3 days

Comments: cloudy day 3 (leaks; media gone)

Initial:		Final:	
FW (g)	6.0	FW	n/d
DW (g)	0.390	DW	n/d
volume (L)	0.750	volume	n/d
conductivity (mS/cm)	3990	conductivity	n/d
packing fraction	0.193	packing fraction	n/d

Reactor #	18A
Date	May 16, 2002
Inoculum and age	6.0 g FW T16, 14d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, humidified room air, NaOH trap at vent
Misting cycle	15 min on / 15 min off
Culture time	4.94 days

Comments: leaks, pressure problems, reservoir top blew off day 5

Initial:		Final:	
FW (g)	6.0	FW	8.63
DW (g)	0.390	DW	0.6730
volume (L)	0.750	volume	0.229
conductivity (mS/cm)	3960	conductivity	3900
packing fraction	0.193	packing fraction	0.278

sugar concentration (g/L)	fructose	glucose	sucrose	glucose equivalents
initial	0	0	30	31.5789
final	7.5797	6.4468	17.1986	32.1304

phosphate (g/L)	
initial	0.1033
final	0.0683

****

Reactor #	19A
Date	May 31, 2002
Inoculum and age	12.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	10.3 days

Comments: clean; early leaks

Initial:		Final:	
FW (g)	12.0	FW	23.75
DW (g)	0.780	DW	2.3097
volume (L)	1.450	conductivity	3860
packing fraction	0.387	packing fraction	0.709

sugar concentration (g/L)	fructose	glucose	sucrose	glucose equivalents
initial	0	0	30	31.5789
final	11.5935	9.7933	10.3935	32.3273

phosphate (g/L)	
initial	0.1033
final	0.0619

Reactor #	19B
Date	May 31, 2002
Inoculum and age	6.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	3 days

Comments: cloudy day 3; malfunctioning flowmeter (airflow too high)

Initial:		Final:	
FW (g)	6.0	FW	n/d
DW (g)	0.390	DW	n/d
volume (L)	1.450	volume	n/d
conductivity (mS/cm)	n/d	conductivity	n/d
packing fraction	0.193	packing fraction	n/d

****

Reactor #	20A
Date	June 21, 2002
Inoculum and age	12.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	6.01 days

Comments: cloudy day 6; humidifier tubing incorrectly configured; humidifier malfunction

Initial:		Final:	
FW (g)	12.0	FW	13.87
DW (g)	0.780	DW	1.0920
volume (L)	1.500	volume	0.710
conductivity (mS/cm)	3860	conductivity	4150
packing fraction	0.387	packing fraction	0.414

Time (days)	Volume change (mL)
0.7500	-200
1.7500	-470
2.7500	-655
3.7604	-780

Reactor #	20B
Date	June 21, 2002
Inoculum and age	9.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	6.03 days

Comments: clean

Initial:		Final:	
FW (g)	9.0	FW	13.85
DW (g)	0.585	DW	1.1902
volume (L)	1.125	volume	0.850
conductivity (mS/cm)	3860	conductivity	3840
packing fraction	0.290	packing fraction	0.447

sugar concentration (g/L)	fructose	glucose	sucrose	glucose equivalents
initial	0	0	30	31.5789
final	6.1777	5.1378	19.096	31.4166

phosphate (g/L)	
initial	0.1033
final	0.0789

Reactor #	21B
Date	July 4, 2002
Inoculum and age	9.0 g FW T16, 14d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	6.01 days
Comments: clean	

Initial:		Final:	
FW (g)	9.0	FW	13.72
DW (g)	0.585	DW	1.2968
volume (L)	1.125	volume	0.950
conductivity (mS/cm)	3460	conductivity	3390
packing fraction	0.290	packing fraction	0.410

Time (days)	Volume change (mL)
1.0000	-10
2.0521	-45
3.1875	-80
4.0208	-110
5.0104	-140
6.0104	-170

sugar concentration (g/L)	fructose	glucose	sucrose	glucose equivalents
initial	0	0	30	31.5789
final	7.4168	6.3692	17.0038	31.6847

phosphate (g/L)	
initial	0.1033
final	0.0747

Reactor #	22A
Date	July 12, 2002
Inoculum and age	12.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	6 days

Comments: clean, but humidifier flooded and died day 3; replaced

Initial:		Final:	
FW (g)	12.0	FW	16.53
DW (g)	0.780	DW	1.5406
volume (L)	1.500	volume	1.280
conductivity (mS/cm)	3140	conductivity	n/d
packing fraction	0.387	packing fraction	0.493

Time (days)	Volume change (mL)
0.8750	-95
2.1146	-130
2.8542	-145
3.8958	-160
4.8542	-175
5.9375	-205

sugar concentration (g/L)	fructose	glucose	sucrose	glucose equivalents
initial	0	0	30	31.5789
final	5.4348	4.6851	21.0774	32.3066

phosphate (g/L)	
initial	0.1033
final	0.0797

Reactor #	22B
Date	July 12, 2002
Inoculum and age	6.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	5 days

Comments: leaks; media ran out; slightly cloudy

Initial:		Final:	
FW (g)	6.0	FW	7.19
DW (g)	0.390	DW	0.6508
volume (L)	0.750	volume	0.143
conductivity (mS/cm)	3710	conductivity	3990
packing fraction	0.193	packing fraction	0.232

Time (days)	Volume change (mL)
0.8750	-120
2.1146	-250
2.8542	-320
3.8958	-395
4.8542	-535

sugar concentration (g/L)	fructose	glucose	sucrose	glucose equivalents
initial	0	0	30	31.5789
final	8.5335	6.1521	17.0218	32.6032

phosphate (g/L)	
initial	0.1033
final	0.0807

Reactor #	23A
Date	July 20, 2002
Inoculum and age	12.0 g FW T16, 16d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	6.01 days

Comments: leaks below growth chamber, but clean

Initial:		Final:		
FW (g)	12.0	FW	17.30	non-blotted bed weight (g)
DW (g)	0.780	DW	1.6919	
volume (L)	1.500	volume	0.685	
conductivity (mS/cm)	3670	conductivity	3630	
packing fraction	0.387	packing fraction	0.516	

Time (days)	Volume change (mL)
1.1771	-405
2.0104	-520
3.1146	-545
4.0729	-785
4.9688	-805
6.0104	-835

sugar concentration (g/L)	fructose	glucose	sucrose	glucose equivalents
initial	0	0	30	31.5789
final	7.4101	6.4431	18.6437	33.4782

phosphate (g/L)	
initial	0.1033
final	0.0825

Reactor #	24A
Date	August 2, 2002
Inoculum and age	6.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	6.01 days

Comments: clean; small leaks

Initial:		Final:	
FW (g)	6.0	FW	8.36
DW (g)	0.390	DW	0.8168
volume (L)	0.750	volume	0.515
conductivity (mS/cm)	3650	conductivity	3860
packing fraction	0.193	packing fraction	0.270

Time (days)	Volume change (mL)
1.0104	-50
2.1354	-120
2.9063	-155
3.9167	-180
5.0104	-195
5.9583	-225

sugar concentration (g/L)	fructose	glucose	sucrose	glucose equivalents
initial	0	0	30	31.5789
final	7.0981	6.1812	20.3752	34.7268

phosphate (g/L)	
initial	0.1033
final	0.0878

Reactor #	24B
Date	August 2, 2002
Inoculum and age	6.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	6 days

Comments: cloudy day 4

Initial:		Final:	
FW (g)	6.0	FW	8.20
DW (g)	0.390	DW	0.7454
volume (L)	0.750	volume	0.460
conductivity (mS/cm)	3650	conductivity	3880
packing fraction	0.193	packing fraction	0.265

Time (days)	Volume change (mL)
1.0104	-60
2.1354	-115
2.9063	-140
3.9167	-170
5.0104	-190
5.9583	-250

sugar concentration (g/L)	fructose	glucose	sucrose	glucose equivalents
initial	0	0	30	31.5789
final	9.0338	7.2174	14.6842	31.7083

phosphate (g/L)	
initial	0.1033
final	0.0743

****

Reactor #	25A
Date	August 10, 2002
Inoculum and age	9.0 g FW T16, 16d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	4 days

Comments: cloudy day 4

Initial:		Final:	
FW (g)	9.0	FW	n/d
DW (g)	0.585	DW	n/d
volume (L)	1.125	volume	n/d
conductivity (mS/cm)	n/d	conductivity	n/d
packing fraction	0.290	packing fraction	n/d

Reactor #	25B
Date	August 10, 2002
Inoculum and age	9.0 g FW T16, 16d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	6 days

Comments: cloudy day 6

Initial:		Final:	
FW (g)	9.0	FW	12.05
DW (g)	0.585	DW	1.0628
volume (L)	1.125	volume	0.810
conductivity (mS/cm)	n/d	conductivity	3210
packing fraction	0.290	packing fraction	0.360

Time (days)	Volume change (mL)
1.1458	-100
2.2083	-185
2.9583	-240
3.8958	-280
4.9583	-300
5.8750	-320

sugar concentration (g/L)	fructose	glucose	sucrose	glucose equivalents
initial	0	0	30	31.5789
final	7.2527	5.7351	16.7981	30.6699

phosphate (g/L)	
initial	0.1033
final	0.0644

Reactor #	26A
Date	August 23, 2002
Inoculum and age	9.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	4 days

Comments: cloudy day 4

Initial:		Final:	
FW (g)	9.0	FW	n/d
DW (g)	0.585	DW	n/d
volume (L)	1.125	volume	n/d
conductivity (mS/cm)	3990	conductivity	n/d
packing fraction	0.290	packing fraction	n/d

****

Reactor #	26B
Date	August 23, 2002
Inoculum and age	6.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	3 days

Comments: cloudy day 3

Initial:		Final:	
FW (g)	6.0	FW	n/d
DW (g)	0.390	DW	n/d
volume (L)	0.750	volume	n/d
conductivity (mS/cm)	3990	conductivity	n/d
packing fraction	0.193	packing fraction	n/d

****

Reactor #	27A
Date	August 30, 2002
Inoculum and age	9.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	2 days

Comments: media gone day 2

Initial:		Final:	
FW (g)	9.0	FW	n/d
DW (g)	0.585	DW	n/d
volume (L)	1.125	volume	n/d
conductivity (mS/cm)	4000	conductivity	n/d
packing fraction	0.290	packing fraction	n/d

Reactor #	27B
Date	August 30, 2002
Inoculum and age	6.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	6 days

Comments: clean

Initial:		Final:	
FW (g)	6.0	FW	9.04
DW (g)	0.390	DW	0.8217
volume (L)	0.750	volume	0.390
conductivity (mS/cm)	4000	conductivity	3880
packing fraction	0.193	packing fraction	0.270

Time (days)	Volume change (mL)
1.1250	-95
2.8958	-230
3.9583	-300
4.9167	-325
5.8875	-350

sugar concentration (g/L)	fructose	glucose	sucrose	glucose equivalents
initial	0	0	30	31.5789
final	6.9738	5.8358	17.3319	31.0537

phosphate (g/L)	
initial	0.1033
final	0.0772

Reactor #	28A
Date	September 13, 2002
Inoculum and age	9.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	6 days

Comments: leaks; humidifier problems (changed day 0 and day 4)

Initial:		Final:	
FW (g)	9.0	FW	13.40
DW (g)	0.585	DW	1.2778
volume (L)	1.125	volume	0.845
conductivity (mS/cm)	3820	conductivity	3730
packing fraction	0.290	packing fraction	0.400

Time (days)	Volume change (mL)
1.1701	-120
3.0451	-170
3.9271	-220

sugar concentration (g/L)	fructose	glucose	sucrose	glucose equivalents
initial	0	0	30	31.5789
final	8.4546	7.224	15.6194	32.1200

phosphate (g/L)	
initial	0.1033
final	0.0729

Reactor #	28B
Date	September 13, 2002
Inoculum and age	9.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	3 days

Comments: leaks; cloudy day 3

Initial:		Final:	
FW (g)	9.0	FW	n/d
DW (g)	0.585	DW	n/d
volume (L)	1.125	volume	n/d
conductivity (mS/cm)	3820	conductivity	n/d
packing fraction	0.290	packing fraction	n/d

****

Reactor #	29A
Date	September 27, 2002
Inoculum and age	12.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	3 days

Comments: cloudy day 3

Initial:		Final:	
FW (g)	12.0	FW	n/d
DW (g)	0.780	DW	n/d
volume (L)	1.500	volume	n/d
conductivity (mS/cm)	3790	conductivity	n/d
packing fraction	0.387	packing fraction	n/d

Reactor #	30A
Date	October 11, 2002
Inoculum and age	12.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	2 days

Comments: cloudy day 2

Initial:		Final:	
FW (g)	12.0	FW	n/d
DW (g)	0.780	DW	n/d
volume (L)	1.500	volume	n/d
conductivity (mS/cm)	3780	conductivity	n/d
packing fraction	0.387	packing fraction	n/d

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Reactor #	30B
Date	October 11, 2002
Inoculum and age	none
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	3 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	days

Comments: air flow problems; generator leaks into humidifier; humidifier left off; leaks below growth chamber; blue dye in media

Initial:		Final:	
FW (g)	0	FW	n/d
DW (g)	0	DW	n/d
volume (L)	1.500	volume	n/d
conductivity (mS/cm)	3780	conductivity	n/d
packing fraction	0	packing fraction	n/d

Reactor #	31B
Date	October 26, 2002
Inoculum and age	12.0 g FW T16, 16d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	6 days

Comments: small fungus in media reservoir at harvest; otherwise clear; red dye in media

Initial:		Final:	
FW (g)	12.0	FW	17.35
DW (g)	0.585	DW	1.6656
volume (L)	1.500	volume	1.320
conductivity (mS/cm)	3920	conductivity	3840
packing fraction	0.387	packing fraction	0.518

Time (days)	Volume change (mL)
2.0833	-20
3.0903	-50
4.0694	-90
5.0972	-115

sugar concentration (g/L)	fructose	glucose	sucrose	glucose equivalents
initial	0	0	30	31.5789
final	7.7756	6.6566	16.3073	31.5978

phosphate (g/L)	
initial	0.1033
final	0.0775

Reactor #	32A
Date	November 2, 2002
Inoculum and age	9.0 g FW T16, 16d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	6 days

Comments: humidifier problems (floods; not misting); red dye in media

Initial:		Final:	
FW (g)	9.0	FW	12.31
DW (g)	0.585	DW	1.1709
volume (L)	1.125	volume	0.790
conductivity (mS/cm)	n/d	conductivity	n/d
packing fraction	0.290	packing fraction	0.367

Time (days)	Volume change (mL)
1.2292	-115
2.0313	-155
2.8542	-165
3.8472	-210
4.9722	-260
5.9618	-285

sugar concentration (g/L)	fructose	glucose	sucrose	glucose equivalents
initial	0	0	30	31.5789
final	6.2046	5.4536	19.1657	31.8326

phosphate (g/L)	
initial	0.1033
final	0.0779

Reactor #	33B
Date	November 9, 2002
Inoculum and age	6.0 g FW T16, 16d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	days

Comments: pump controller died three times; not misting; red dye in media

Initial:		Final:	
FW (g)	6.0	FW	n/d
DW (g)	0.390	DW	n/d
volume (L)	0.750	volume	n/d
conductivity (mS/cm)	n/d	conductivity	n/d
packing fraction	0.193	packing fraction	n/d

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Reactor #	34A
Date	November 23, 2002
Inoculum and age	9.0 g FW T16, 16d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	3 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	6 days

Comments: clean; red dye in media

Initial:		Final:			
FW (g)	9.0	FW	12.44	non-blotted bed weight	18.88
DW (g)	0.585	DW	1.1853		
volume (L)	1.125	volume	0.970		
conductivity (mS/cm)	n/d	conductivity	n/d		
packing fraction	0.290	packing fraction	0.401		

Time (days)	Volume change (mL)
0.9583	-45
1.9271	-50
2.9792	-75
3.9792	-100
4.8542	-115
5.9583	-130

Reactor #	34B
Date	November 23, 2002
Inoculum and age	6.0 g FW T16, 16d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	3 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	3 days

Comments: cloudy day 3; red dye in media

Initial:		Final:	
FW (g)	6.0	FW	n/d
DW (g)	0.390	DW	n/d
volume (L)	0.750	volume	n/d
conductivity (mS/cm)	n/d	conductivity	n/d
packing fraction	0.193	packing fraction	n/d

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Reactor #	35A
Date	December 6, 2002
Inoculum and age	9.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	6 days

Comments: humidifier flooded, shorted out from overflow; replaced; flooded~~.~~ split tubing from mist generator bubble aspirator; red dye in media

Initial:		Final:			
FW (g)	9.0	FW	11.33	non-blotted bed weight	17.39
DW (g)	0.585	DW	1.0111		
volume (L)	1.125	volume	n/d		
conductivity (mS/cm)	3710	conductivity	3860		
packing fraction	0.290	packing fraction	0.338		

Reactor #	35B
Date	December 6, 2002
Inoculum and age	6.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	2.5 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	6 days

Comments: mist problems day 3; air pump dying– replaced day 6; red dye in media

Initial:		Final:			
FW (g)	6.0	FW	8.65	non-blotted bed weight	12.87
DW (g)	0.390	DW	0.7197		
volume (L)	0.750	volume	0.655		
conductivity (mS/cm)	3710	conductivity	3590		
packing fraction	0.193	packing fraction	0.258		

Time (days)	Volume change (mL)
1.1146	-15
2.8750	-45
3.9583	-50
4.875	-80
5.9858	-85

sugar concentration (g/L)	fructose	glucose	sucrose	glucose equivalents
initial	0	0	30	31.5789
final	2.0565	1.7269	25.2983	30.4132

phosphate (g/L)	
initial	0.1033
final	0.0957

Reactor #	36B
Date	December 14, 2002
Inoculum and age	9.0 g FW T16, 16d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	6 days

Comments: left humidifier off day 45; cloudy day 6; red dye in media

Initial:		Final:			
FW (g)	9.0	FW	12.44	non-blotted bed weight	19.39
DW (g)	0.585	DW	1.1470		
Volume (L)	1.125	volume	0.950		
conductivity (mS/cm)	3870	conductivity	3790		
packing fraction	0.290	packing fraction	0.371		

Time (days)	Volume change (mL)
1.0417	-20
2.0313	-55
2.9167	-65
4.0104	-125
4.9583	-125
5.9896	-155

sugar concentration (g/L)	fructose	glucose	sucrose	glucose equivalents
initial	0	0	30	31.5789
final	5.1387	4.2454	20.9546	31.4416

phosphate (g/L)	
initial	0.1033
final	0.0832

Reactor #	37A
Date	December 28, 2002
Inoculum and age	9.0 g FW T16, 16d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	6 days

Comments: kinked tubing to mist generator day 3 (not misting); cloudy day 5; red dye in media

Initial:		Final:			
FW (g)	9.0	FW	11.90	non-blotted bed weight	17.69
DW (g)	0.585	DW	1.0574		
volume (L)	1.125	volume	0.595		
conductivity (mS/cm)	3870	conductivity	4010		
packing fraction	0.290	packing fraction	0.355		

Time (days)	Volume change (mL)
1.9688	-115
2.9931	-115
5.0104	-390
6.0035	-485

sugar concentration (g/L)	fructose	glucose	sucrose	glucose equivalents
initial	0	0	30	31.5789
final	8.1707	6.3838	15.1805	30.5340

phosphate (g/L)	
initial	0.1033
final	0.0804

Reactor #	37B
Date	December 28, 2002
Inoculum and age	6.0 g FW T16, 16d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	6 days

Comments: cloudy day 5; red dye in media

Initial:		Final:			
FW (g)	6.0	FW	8.86	non-blotted bed weight	13.41
DW (g)	0.390	DW	0.7822		
volume (L)	0.750	volume	0.580		
conductivity (mS/cm)	3870	conductivity	4080		
packing fraction	0.193	packing fraction	0.285		

Time (days)	Volume change (mL)
1.9688	-100
2.9931	-130
5.0104	-160
6.0035	-180

sugar concentration (g/L)	fructose	glucose	sucrose	glucose equivalents
initial	0	0	30	31.5789
final	7.0423	5.4193	17.5037	30.8865

phosphate (g/L)	
initial	0.1033
final	0.0789

Reactor #	38A
Date	January 11, 2003
Inoculum and age	12.0 g FW T16, 16d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	2 days

Comments: cloudy day 2 (media sterile filtration problems)

Initial:		Final:	
FW (g)	12.0	FW	n/d
DW (g)	0.780	DW	n/d
volume (L)	1.500	volume	n/d
conductivity (mS/cm)	n/d	conductivity	n/d
packing fraction	0.387	packing fraction	n/d

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Reactor #	38B
Date	January 11, 2003
Inoculum and age	6.0 g FW T16, 16d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	6 days

Comments: clean

Initial:		Final:			
FW (g)	6.0	FW	8.14	non-blotted bed weight	13.05
DW (g)	0.390	DW	0.7352		
volume (L)	0.750	volume	0.620		
conductivity (mS/cm)	n/d	conductivity	3660		
packing fraction	0.193	packing fraction	0.263		

sugar concentration (g/L)	fructose	glucose	sucrose	glucose equivalents
initial	0	0	30	31.5789
final	4.8781	4.1223	21.5233	31.6566

phosphate (g/L)	
initial	0.1033
final	0.0768

Reactor #	39A
Date	January 17, 2003
Inoculum and age	9.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	6 days

Comments: not misting day 4- replaced humidifier; slightly cloudy media at harvest

Initial:		Final:			
FW (g)	9.0	FW	13.03	non-blotted bed weight	19.52
DW (g)	0.585	DW	1.1805		
volume (L)	1.125	volume	0.865		
conductivity (mS/cm)	3760	conductivity	3290		
packing fraction	0.290	packing fraction	0.404		

Time (days)	Volume change (mL)
1.0486	-60
2.0243	-115
3.0938	-145
4.3646	-190

sugar concentration (g/L)	fructose	glucose	sucrose	glucose equivalents
initial	0	0	30	31.5789
final	5.8496	4.7804	18.4224	30.0220

phosphate (g/L)	
initial	0.1033
final	0.0732

Reactor #	40B
Date	January 25, 2002
Inoculum and age	9.0 g FW T16, 16d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	6 days

Comments: slightly cloudymedia at harvest; temp problems in culture room (cold)

Initial:		Final:			
FW (g)	9.0	FW	10.11	non-blotted bed weight	16.13
DW (g)	0.585	DW	0.9334		
volume (L)	1.125	volume	0.950		
conductivity (mS/cm)	3840	conductivity	4020		
packing fraction	0.290	packing fraction	0.326		

Time (days)	Volume change (mL)
1.17361111	-35
2.13888889	-75
4.05555556	-120
5.01388889	-120

sugar concentration (g/L)	fructose	glucose	sucrose	glucose equivalents
initial	0	0	30	31.5789
final	6.4484	4.9407	18.0349	30.3732

phosphate (g/L)	
initial	0.1033
final	0.0868

Reactor #	41A
Date	January 31, 2003
Inoculum and age	9.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	3.02 days

Comments: not misting day 1– media feed problems; generator vent not clamped– lost media

Initial:		Final:			
FW (g)	9.0	FW	n/d	non-blotted bed weight	12.20
DW (g)	0.585	DW	0.7602		
volume (L)	1.125	volume	n/d		
conductivity (mS/cm)	3820	conductivity	n/d		
packing fraction	0.290	packing fraction	n/d		

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Reactor #	42A
Date	February 9, 2003
Inoculum and age	9.0 g FW T16, 17d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	1 day

Comments: possibly leaking into humidifier day 1; media feed problems– burned out mist generator membrane

Initial:		Final:	
FW (g)	9.0	FW	n/d
DW (g)	0.585	DW	n/d
volume (L)	1.125	volume	n/d
conductivity (mS/cm)	3800	conductivity	n/d
packing fraction	0.290	packing fraction	n/d

Reactor #	42B
Date	February 9, 2003
Inoculum and age	9.0 g FW T16, 17d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	2 days

Comments: not misting well day 1; changed humidifier

Initial:		Final:			
FW (g)	9.0	FW	n/d	non-blotted bed weight	11.36
DW (g)	0.585	DW	0.7433		
volume (L)	1.125	volume	n/d		
conductivity (mS/cm)	3800	conductivity	n/d		
packing fraction	0.290	packing fraction	n/d		

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Reactor #	43B
Date	February 14, 2003
Inoculum and age	9.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	2 days

Comments: blotted/weighed roots sitting for 2 hr; had to resterilize reactor; otherwise, clean

Initial:		Final:			
FW (g)	9.0	FW	n/d	non-blotted bed weight	9.79
DW (g)	0.585	DW	0.6196		
volume (L)	1.125	volume	0.580		
conductivity (mS/cm)	3750	conductivity	3740		
packing fraction	0.290	packing fraction	n/d		

sugar concentration (g/L)	fructose	glucose	sucrose	glucose equivalents
initial	0	0	30	31.5789
final	0.8383	0.611	25.8482	28.6579

phosphate (g/L)	
initial	0.1033
final	0.0932

Reactor #	44A
Date	February 21, 2003
Inoculum and age	9.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	2 days

Comments: clean

Initial:		Final:			
FW (g)	9.0	FW	n/d	non-blotted bed weight	12.63
DW (g)	0.585	DW	0.8264		
volume (L)	1.125	volume	1.050		
conductivity (mS/cm)	3750	conductivity	3620		
packing fraction	0.290	packing fraction	n/d		

Time (days)	Volume change (mL)
0.8750	-20
2.0000	-55

sugar concentration (g/L)	fructose	glucose	sucrose	glucose equivalents
initial	0	0	30	31.5789
final	1.4196	1.1887	26.7649	30.7819

phosphate (g/L)	
initial	0.1033
final	0.0960

Reactor #	44B
Date	February 21, 2003
Inoculum and age	9.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	2 days
Comments: clean	

Initial:		Final:			
FW (g)	9.0	FW	n/d	non-blotted bed weight	12.75
DW (g)	0.585	DW	0.8476		
volume (L)	1.125	volume	1.050		
conductivity (mS/cm)	3800	conductivity	3620		
packing fraction	0.290	packing fraction	n/d		

Time (days)	Volume change (mL)
0.8750	-35
2.0000	-75

sugar concentration (g/L)	fructose	glucose	sucrose	glucose equivalents
initial	0	0	30	31.5789
final	1.3716	1.2172	27.0469	31.0591

phosphate (g/L)	
initial	0.1033
final	0.0985

Reactor #	45B
Date	February 28, 2003
Inoculum and age	9.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	2 days
Comments: clean	

Initial:		Final:			
FW (g)	9.0	FW	n/d	non-blotted bed weight	12.24
DW (g)	0.585	DW	0.7774		
volume (L)	1.125	volume	1.080		
conductivity (mS/cm)	3800	conductivity	3720		
packing fraction	0.290	packing fraction	n/d		

Time (days)	Volume change (mL)
0.9861	-40

sugar concentration (g/L)	fructose	glucose	sucrose	glucose equivalents
initial	0	0	30	31.5789
final	1.0932	0.9656	25.6332	29.0411

phosphate (g/L)	
initial	0.1033
final	0.0896

Reactor #	46A
Date	March 7, 2003
Inoculum and age	9.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	4 days

Comments: 2 small fungal colonies in reservoir at harvest

Initial:		Final:			
FW (g)	9.0	FW	n/d	non-blotted bed weight	15.96
DW (g)	0.585	DW	1.1260		
volume (L)	1.125	volume	0.915		
conductivity (mS/cm)	3730	conductivity	3570		
packing fraction	0.290	packing fraction	n/d		

Time (days)	Volume change (mL)
1.2292	-30
2.9792	-110

sugar concentration (g/L)	fructose	glucose	sucrose	glucose equivalents
initial	0	0	30	31.5789
final	4.141	3.551	21.9304	30.7766

phosphate (g/L)	
initial	0.1033
final	0.0864

Reactor #	46B
Date	March 8, 2003
Inoculum and age	9.0 g FW T16, 16d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	4 days

Comments: fungal colonies in media at harvest

Initial:		Final:			
FW (g)	9.0	FW	n/d	non-blotted bed weight	17.10
DW (g)	0.585	DW	1.1251		
volume (L)	1.125	volume	n/d		
conductivity (mS/cm)	3730	conductivity	3800		
packing fraction	0.290	packing fraction	n/d		

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Reactor #	47A
Date	March 14, 2003
Inoculum and age	9.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	days

Comments: cloudy at harvest; too warm in culture room

Initial:		Final:			
FW (g)	9.0	FW	n/d	non-blotted bed weight	15.3
DW (g)	0.585	DW	1.040		
volume (L)	1.125	volume	0.915		
conductivity (mS/cm)	n/d	conductivity	n/d		
packing fraction	0.290	packing fraction	n/d		

Reactor #	48A
Date	March 22, 2003
Inoculum and age	9.0 g FW T16, 16d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	4 days

Comments: slightly cloudy media at harvest

Initial:		Final:			
FW (g)	9.0	FW	n/d	non-blotted bed weight	15.26
DW (g)	0.585	DW	1.1053		
volume (L)	1.125	volume	0.860		
conductivity (mS/cm)	n/d	conductivity	n/d		
packing fraction	0.290	packing fraction	n/d		

Time (days)	Volume change (mL)
0.9896	-45
1.8333	-105
2.8507	-165
3.8958	-235

sugar concentration (g/L)	fructose	glucose	sucrose	glucose equivalents
initial	0	0	30	31.5789
final	4.0963	3.6304	22.5946	31.5104

phosphate (g/L)	
initial	0.1033
final	0.0857

Reactor #	48B
Date	March 22, 2003
Inoculum and age	9.0 g FW T16, 16d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	4 days

Comments: mist generator overfull– not misting day 1

Initial:		Final:			
FW (g)	9.0	FW	n/d	non-blotted bed weight	15.31
DW (g)	0.585	DW	1.1019		
volume (L)	1.125	volume	0.980		
conductivity (mS/cm)	n/d	conductivity	n/d		
packing fraction	0.290	packing fraction	n/d		

Time (days)	Volume change (mL)
1.8333	-90
2.8507	-155
3.8958	-155

sugar concentration (g/L)	fructose	glucose	sucrose	glucose equivalents
initial	0	0	30	31.5789
final	3.2183	2.6243	25.2533	32.4251

phosphate (g/L)	
initial	0.1033
final	0.0818

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Reactor #	49A
Date	March 28, 2003
Inoculum and age	9.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 5% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	6 days

Comments: brown media day 4; brown roots day 5

Initial:		Final:			
FW (g)	9.0	FW	n/d	non-blotted bed weight	11.52
DW (g)	0.585	DW	n/d		
volume (L)	1.125	volume	0.530		
conductivity (mS/cm)	3610	conductivity	3920		
packing fraction	0.290	packing fraction	n/d		

Reactor #	49B
Date	March 28, 2003
Inoculum and age	9.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 5% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	6 days
Comments: clean	

Initial:		Final:			
FW (g)	9.0	FW	n/d	non-blotted bed weight	21.75
DW (g)	0.585	DW	2.0507		
volume (L)	1.125	volume	0.930		
conductivity (mS/cm)	3610	conductivity	3560		
packing fraction	0.290	packing fraction	n/d		

Time (days)	Volume change (mL)
1.1354	-115
1.8958	-115
2.8368	-140
3.9132	-180
4.8472	-215

sugar concentration (g/L)	fructose	glucose	sucrose	glucose equivalents
initial	0	0	50	52.6316
final	8.5234	6.9048	39.2544	56.7485

phosphate (g/L)	
initial	0.1033
final	0.0747

Reactor #	50B
Date	April 11, 2003
Inoculum and age	9.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 5% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	6 days
Comments: clean	

Initial:		Final:			
FW (g)	9.0	FW	n/d	non-blotted bed weight	20.37
DW (g)	0.585	DW	1.6970		
volume (L)	1.125	volume	0.895		
conductivity (mS/cm)	3580	conductivity	3520		
packing fraction	0.290	packing fraction	n/d		

Time (days)	Volume change (mL)
1.1632	-105
2.0278	-170
3.8576	-210
5.1875	-210
5.8646	-250

sugar concentration (g/L)	fructose	glucose	sucrose	glucose equivalents
initial	0	0	50	52.6316
final	9.1597	7.9788	37.8718	57.0035

phosphate (g/L)	
initial	0.1033
final	0.0711

Reactor #	51A
Date	April 23, 2003
Inoculum and age	9.0 g FW T16, 13d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 5% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	2 days

Comments: not misting well @7 hours– fixed; clean at harvest

Initial:		Final:			
FW (g)	9.0	FW	n/d	non-blotted bed weight	11.63
DW (g)	0.585	DW	0.8013		
volume (L)	1.125	volume	1.020		
conductivity (mS/cm)	3640	conductivity	3470		
packing fraction	0.290	packing fraction	n/d		

Time (days)	Volume change (mL)
0.1042	0
0.8333	-70
1.8125	-85

sugar concentration (g/L)	fructose	glucose	sucrose	glucose equivalents
initial	0	0	50	52.6316
final	1.4092	1.2104	46.0214	51.0632

phosphate (g/L)	
initial	0.1033
final	0.0893

Reactor #	51B
Date	April 23, 2003
Inoculum and age	9.0 g FW T16, 13d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 5% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	21 days

Comments: overfull mist generator day 1; humidifier level too low day 4; dark media day 15; brown/fungus roots day 21

Initial:		Final:			
FW (g)	9.0	FW	n/d	non-blotted bed weight	18.79
DW (g)	0.585	DW	1.9098		
volume (L)	1.125	volume	0.352		
conductivity (mS/cm)	3640	conductivity	5390		
packing fraction	0.290	packing fraction	n/d		

sugar concentration (g/L)	fructose	glucose	sucrose	glucose equivalents
initial	0	0	50	52.6316
final	36.1637	27.4263	2.7748	66.5109

phosphate (g/L)	
initial	0.1033
final	0.1668

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Reactor #	52A
Date	May 2, 2003
Inoculum and age	9.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 5% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	1 day

Comments: split tubing from mist generator oveflow – lost media day 1

Initial:		Final:	
FW (g)	9.0	FW	n/d
DW (g)	0.585	DW	n/d
volume (L)	1.125	volume	n/d
conductivity (mS/cm)	3710	conductivity	n/d
packing fraction	0.290	packing fraction	n/d

Reactor #	53A
Date	May 16, 2003
Inoculum and age	9.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	4 days
Comments: clean	

Initial:		Final:			
FW (g)	9.0	FW	n/d	non-blotted bed weight	17.44
DW (g)	0.585	DW	1.2745		
volume (L)	1.125	volume	1.000		
conductivity (mS/cm)	3620	conductivity	3460		
packing fraction	0.290	packing fraction	n/d		

Time (days)	Volume change (mL)
0.9722	-60
2.8889	-125

sugar concentration (g/L)	fructose	glucose	sucrose	glucose equivalents
initial	0	0	30	31.5789
final	4.469	3.9226	40.6994	51.2330

phosphate (g/L)	
initial	0.1033
final	0.0804

Reactor #	53B
Date	May 16, 2003
Inoculum and age	9.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 5% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	6 days

Comments: cloudy at harvest

Initial:		Final:			
FW (g)	9.0	FW	n/d	non-blotted bed weight	19.17
DW (g)	0.585	DW	n/d		
volume (L)	1.125	volume	0.815		
conductivity (mS/cm)	3620	conductivity	3420		
packing fraction	0.290	packing fraction	n/d		

Time (days)	Volume change (mL)
0.9722	-100
2.8889	-195
3.9410	-225
4.8681	-300

sugar concentration (g/L)	fructose	glucose	sucrose	glucose equivalents
initial	0	0	50	52.6316
final	10.3108	9.2398	45.2948	67.2293

phosphate (g/L)	
initial	0.1033
final	0.0736

Reactor #	54A
Date	May 30, 2003
Inoculum and age	9.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 5% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	2 days
Comments: clean	

Initial:		Final:			
FW (g)	9.0	FW	n/d	non-blotted bed weight	14.19
DW (g)	0.585	DW	0.8300		
volume (L)	1.125	volume	0.975		
conductivity (mS/cm)	3400	conductivity	3300		
packing fraction	0.290	packing fraction	n/d		

Time (days)	Volume change (mL)
1.0660	-30
1.9826	-90

sugar concentration (g/L)	fructose	glucose	sucrose	glucose equivalents
initial	0	0	50	52.6316
final	1.4629	1.3101	47.5511	52.8269

phosphate (g/L)	
initial	0.1033
final	0.0921

Reactor #	54B
Date	May 30, 2003
Inoculum and age	9.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 5% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	4 days

Comments: cloudy at harvest

Initial:		Final:			
FW (g)	9.0	FW	n/d	non-blotted bed weight	16.67
DW (g)	0.585	DW	1.1425		
volume (L)	1.125	volume	0.975		
conductivity (mS/cm)	3400	conductivity	3380		
packing fraction	0.290	packing fraction	n/d		

Time (days)	Volume change (mL)
1.0660	-90
1.9826	-120
3.8229	-200

sugar concentration (g/L)	fructose	glucose	sucrose	glucose equivalents
initial	0	0	50	52.6316
final	3.9236	3.4128	43.7637	53.4035

phosphate (g/L)	
initial	0.1033
final	0.0850

Reactor #	55A
Date	June 6, 2003
Inoculum and age	9.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 5% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	4 days

Comments: slightly cloudy at harvest

Initial:		Final:			
FW (g)	9.0	FW	n/d	non-blotted bed weight	16.38
DW (g)	0.585	DW	1.1396		
volume (L)	1.125	volume	0.875		
conductivity (mS/cm)	3340	conductivity	3360		
packing fraction	0.290	packing fraction	n/d		

Time (days)	Volume change (mL)
2.8090	-155
3.8472	-235

sugar concentration (g/L)	fructose	glucose	sucrose	glucose equivalents
initial	0	0	50	52.6316
final	4.7614	4.1206	41.437	52.4998

phosphate (g/L)	
initial	0.1033
final	0.0797

Reactor #	55B
Date	June 6, 2003
Inoculum and age	9.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 5% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	4 days
Comments: clean	

Initial:		Final:			
FW (g)	9.0	FW	n/d	non-blotted bed weight	17.19
DW (g)	0.585	DW	1.3061		
volume (L)	1.125	volume	1.020		
conductivity (mS/cm)	3340	conductivity	3290		
packing fraction	0.290	packing fraction	n/d		

Time (days)	Volume change (mL)
1.0417	-70
2.8090	-130
3.8472	-145

sugar concentration (g/L)	fructose	glucose	sucrose	glucose equivalents
initial	0	0	50	52.6316
final	4.0514	3.8327	43.8047	53.9943

phosphate (g/L)	
initial	0.1033
final	0.0868

Reactor #	56B
Date	June 13, 2003
Inoculum and age	9.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 5% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	4 days

Comments: some leaks; small fungal colonies in reservoir at harvest

Initial:		Final:			
FW (g)	9.0	FW	n/d	non-blotted bed weight	15.25
DW (g)	0.585	DW	1.0663		
volume (L)	1.125	volume	1.000		
conductivity (mS/cm)	3340	conductivity	3200		
packing fraction	0.290	packing fraction	n/d		

Time (days)	Volume change (mL)
1.1563	-65
3.0694	-95
3.9792	-95

sugar concentration (g/L)	fructose	glucose	sucrose	glucose equivalents
initial	0	0	50	52.6316
final	3.7241	3.3367	43.759	53.1229

phosphate (g/L)	
initial	0.1033
final	0.0772

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Reactor #	57A
Date	June 20, 2003
Inoculum and age	0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 5% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	days

Comments: fell over day 1; low humidifier level (not misting); reservoir media mysteriously gone day 3

Initial:		Final:			
FW (g)	9.0	FW	n/d	non-blotted bed weight	13.01
DW (g)	0.585	DW	n/d		
volume (L)	1.125	volume	n/d		
conductivity (mS/cm)	3430	conductivity	3690		
packing fraction	0.290	packing fraction	n/d		

phosphate (g/L)	
initial	0.1033
final	0.0711

Reactor #	57B
Date	June 20, 2003
Inoculum and age	9.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 5% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	6 days

Comments: not misting day 1; small round brown things in media reservoir at harvest

Initial:		Final:			
FW (g)	9.0	FW	n/d	non-blotted bed weight	20.42
DW (g)	0.585	DW	1.6563		
volume (L)	1.125	volume	1.000		
conductivity (mS/cm)	3430	conductivity	3380		
packing fraction	0.290	packing fraction	n/d		

Time (days)	Volume change (mL)
1.1875	-60
2.9097	-85
3.8854	-105
4.8958	-115
5.8750	-170

sugar concentration (g/L)	fructose	glucose	sucrose	glucose equivalents
initial	0	0	50	52.6316
final	7.2347	6.8189	38.1784	54.2414

phosphate (g/L)	
initial	0.1033
final	0.0732

Reactor #	58A
Date	July 3, 2003
Inoculum and age	9.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 5% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	2 days

Comments: not misting day 2, media reservoir low. Weird….

Initial:		Final:	
FW (g)	9.0	FW	n/d
DW (g)	0.585	DW	n/d
volume (L)	1.125	volume	n/d
conductivity (mS/cm)	3400	conductivity	n/d
packing fraction	0.290	packing fraction	n/d

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Reactor #	58B
Date	July 3, 2003
Inoculum and age	9.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 5% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	6 days

Comments: clean

Initial:		Final:			
FW (g)	9.0	FW	n/d	non-blotted bed weight	20.93
DW (g)	0.585	DW	1.6632		
volume (L)	1.125	volume	0.965		
conductivity (mS/cm)	3400	conductivity	3220		
packing fraction	0.290	packing fraction	n/d		

Time (days)	Volume change (mL)
1.9271	-100
3.8681	-120
4.8681	-120
5.9097	-140

sugar concentration (g/L)	fructose	glucose	sucrose	glucose equivalents
initial	0	0	50	52.6316
final	7.5853	6.8673	37.4599	53.8842

phosphate (g/L)	
initial	0.1033
final	0.0683

Reactor #	59A
Date	July 11, 2003
Inoculum and age	9.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 5% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	2 days
Comments: clean	

Initial:		Final:			
FW (g)	9.0	FW	n/d	non-blotted bed weight	12.11
DW (g)	0.585	DW	0.8410		
volume (L)	1.125	volume	1.075		
conductivity (mS/cm)	3470	conductivity	3410		
packing fraction	0.290	packing fraction	n/d		

sugar concentration (g/L)	fructose	glucose	sucrose	glucose equivalents
initial	0	0	50	52.6316
final	1.4681	1.122	47.0611	52.1282

phosphate (g/L)	
initial	0.1033
final	0.0935

Reactor #	60A
Date	July 18, 2003
Inoculum and age	9.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 3 min off
Culture time	6 days

Comments: clean; some mist problems day 0 and 4

Initial:		Final:			
FW (g)	9.0	FW	n/d	non-blotted bed weight	19.77
DW (g)	0.585	DW	1.2949		
volume (L)	1.125	volume	0.860		
conductivity (mS/cm)	3440	conductivity	n/d		
packing fraction	0.290	packing fraction	n/d		

Time (days)	Volume change (mL)
2.0104	-155
2.8646	-175
3.8681	-205
4.9792	-245
5.8750	-270

sugar concentration (g/L)	fructose	glucose	sucrose	glucose equivalents
initial	0	0	30	31.5789
final	7.2131	6.4195	17.2635	31.8047

phosphate (g/L)	
initial	0.1033
final	0.0725

Reactor #	60B
Date	July 18, 2003
Inoculum and age	9.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	10 min on / 10 min off
Culture time	6 days

Comments: clean; mist problems (timer possibly off day θ2)

Initial:		Final:			
FW (g)	9.0	FW	n/d	non-blotted bed weight	17.24
DW (g)	0.585	DW	1.0769		
volume (L)	1.125	volume	1.035		
conductivity (mS/cm)	3440	conductivity	n/d		
packing fraction	0.290	packing fraction	n/d		

Time (days)	Volume change (mL)
2.0104	0
2.8646	-15
3.8681	-50
4.9792	-75
5.8750	-85

sugar concentration (g/L)	fructose	glucose	sucrose	glucose equivalents
initial	0	0	30	31.5789
final	2.98	2.5598	23.7532	30.5433

phosphate (g/L)	
initial	0.1033
final	0.0807

Reactor #	61A
Date	August 1, 2003
Inoculum and age	9.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, humidified roomair
Misting cycle	15 min on / 3 min off
Culture time	6 days

Comments: clean; mist problems; power failure on day 3 may have reset cycle to 15/15 until day 5)

Initial:		Final:			
FW (g)	9.0	FW	n/d	non-blotted bed weight	17.77
DW (g)	0.585	DW	1.2208		
volume (L)	1.125	volume	1.010		
conductivity (mS/cm)	n/d	conductivity	n/d		
packing fraction	0.290	packing fraction	n/d		

Time (days)	Volume change (mL)
1.9271	-15
2.9063	-45
3.9340	-45
4.8889	-75
5.8785	-120

sugar concentration (g/L)	fructose	glucose	sucrose	glucose equivalents
initial	0	0	30	31.5789
final	3.0487	2.5231	21.8839	28.6075

phosphate (g/L)	
initial	0.1033
final	0.0768

Reactor #	61B
Date	August 1, 2003
Inoculum and age	9.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 3 min off
Culture time	6 days

Comments: clean; mist problems day 3

Initial:		Final:			
FW (g)	9.0	FW	n/d	non-blotted bed weight	20.86
DW (g)	0.585	DW	1.3917		
volume (L)	1.125	volume	1.020		
conductivity (mS/cm)	n/d	conductivity	n/d		
packing fraction	0.290	packing fraction	n/d		

Time (days)	Volume change (mL)
1.9271	-45
2.9063	-75
3.9340	-75
4.8889	-110
5.8785	-145

sugar concentration (g/L)	fructose	glucose	sucrose	glucose equivalents
initial	0	0	30	31.5789
final	6.3068	6.0804	17.7287	31.0490

phosphate (g/L)	
initial	0.1033
final	0.0661

Reactor #	62A
Date	August 15, 2003
Inoculum and age	9.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 1.8% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 3 min off
Culture time	6 days

Comments: clean; mist problems day 4

Initial:		Final:			
FW (g)	9.0	FW	n/d	non-blotted bed weight	19.46
DW (g)	0.585	DW	1.0636		
volume (L)	1.125	volume	0.875		
conductivity (mS/cm)	n/d	conductivity	n/d		
packing fraction	0.290	packing fraction	n/d		

Time (days)	Volume change (mL)
0.9514	-45
2.0000	-95
3.9826	-160
5.1563	-195
5.8854	-215

sugar concentration (g/L)	fructose	glucose	sucrose	glucose equivalents
initial	0	0	18	18.9474
final	3.1142	2.2889	10.8986	16.8753

phosphate (g/L)	
initial	0.1033
final	0.0704

Reactor #	62B
Date	August 15, 2003
Inoculum and age	9.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 1.8% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 3 min off
Culture time	days

Comments: clean; mist problems; mystery leak? (media decreased much faster than parallel run 62A)

Initial:		Final:			
FW (g)	9.0	FW	n/d	non-blotted bed weight	17.91
DW (g)	0.585	DW	0.9704		
volume (L)	1.125	volume	0.155		
conductivity (mS/cm)	n/d	conductivity	n/d		
packing fraction	0.290	packing fraction	n/d		

Time (days)	Volume change (mL)
0.9514	-135
2.0000	-320
3.9826	-530
5.1563	-815
5.8854	-960

sugar concentration (g/L)	fructose	glucose	sucrose	glucose equivalents
initial	0	0	18	18.9474
final	2.1803	1.9417	14.7083	19.6043

phosphate (g/L)	
initial	0.1033
final	0.0935

Reactor #	63A
Date	August 29, 2003
Inoculum and age	9.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 1.8% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 3 min off
Culture time	6 days

Comments: mist problems day 2 (humidifier dead- replaced); cloudy

Initial:		Final:			
FW (g)	9.0	FW	n/d	non-blotted bed weight	15.39
DW (g)	0.585	DW	0.7838		
volume (L)	1.125	volume	0.725		
conductivity (mS/cm)	n/d	conductivity	n/d		
packing fraction	0.290	packing fraction	n/d		

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Reactor #	63B
Date	August 29, 2003
Inoculum and age	9.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 1.8% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 3 min off
Culture time	6 days

Comments: mist problems; fungus in roots

Initial:		Final:			
FW (g)	9.0	FW	n/d	non-blotted bed weight	14.46
DW (g)	0.585	DW	0.7378		
volume (L)	1.125	volume	0.885		
conductivity (mS/cm)	n/d	conductivity	n/d		
packing fraction	0.290	packing fraction	n/d		

Reactor #	64A
Date	July 30, 2004
Inoculum and age	9.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 8% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	6 days

Comments: small fungus in media lines at harvest

Initial:		Final:			
FW (g)	9.0	FW	7.17	non-blotted bed weight	10.17
DW (g)	0.585	DW	n/d		
volume (L)	1.125	volume	0.750		
conductivity (mS/cm)	n/d	conductivity	n/d		
packing fraction	0.290	packing fraction	n/d		

Time (days)	Volume change (mL)
1.8854	-225
2.8229	-340
3.8854	-375
4.8854	-425

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Reactor #	64B
Date	August 6, 2004
Inoculum and age	9.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 8% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	0 day

Comments: not misting day 2; no media; weird….

Initial:		Final:	
FW (g)	9.0	FW	n/d
DW (g)	0.585	DW	n/d
volume (L)	1.125	volume	n/d
conductivity (mS/cm)	n/d	conductivity	n/d
packing fraction	0.290	packing fraction	n/d

Reactor #	65A
Date	September 3, 2004
Inoculum and age	9.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 8% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	3 days

Comments: cloudy media day 3

Initial:		Final:	
FW (g)	9.0	FW	n/d
DW (g)	0.585	DW	n/d
volume (L)	1.125	volume	n/d
conductivity (mS/cm)	n/d	conductivity	n/d
packing fraction	0.290	packing fraction	n/d

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Reactor #	65B
Date	September 3, 2004
Inoculum and age	9.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 8% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	6 days

Comments: generators fell over sometime on day 5; media slightly cloudy

Initial:		Final:			
FW (g)	9.0	FW	10.30	non-blotted bed weight	13.52
DW (g)	0.585	DW	1.0919		
volume (L)	1.125	volume	0.815		
conductivity (mS/cm)	n/d	conductivity	n/d		
packing fraction	0.290	packing fraction	n/d		

Time (days)	Volume change (mL)
1.3333	-95
3.1250	-140
3.9201	-175
5.9688	-295

Reactor #	66A
Date	September 24, 2004
Inoculum and age	9.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 5% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 3 min off
Culture time	6 days

Comments: misting problems day 3; media slightly brown day 3; much lower than B; media definitely cloudy day 5

Initial:		Final:			
FW (g)	9.0	FW	n/d	non-blotted bed weight	11.43
DW (g)	0.585	DW	n/d		
volume (L)	1.125	volume	0.395		
conductivity (mS/cm)	n/d	conductivity	n/d		
packing fraction	0.290	packing fraction	n/d		

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Reactor #	66B
Date	September 24, 2004
Inoculum and age	9.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 5% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 3 min off
Culture time	6 days

Comments: misting problems day 4

Initial:		Final:			
FW (g)	9.0	FW	11.3	non-blotted bed weight	13.78
DW (g)	0.585	DW	1.0060		
volume (L)	1.125	volume	0.745		
conductivity (mS/cm)	n/d	conductivity	n/d		
packing fraction	0.290	packing fraction	n/d		

Time (days)	Volume change (mL)
1.9722	-110
2.9896	-225
3.9792	-270
5.0208	-315
5.9792	-365

Reactor #	67A
Date	October 14, 2004
Inoculum and age	9.0 g FW T16, 14d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 5% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	6 days

Comments: reservoir much lower than B; brown and contaminated day 4

Initial:		Final:			
FW (g)	9.0	FW	n/d	non-blotted bed weight	n/d
DW (g)	0.585	DW	n/d		
volume (L)	1.125	volume	n/d		
conductivity (mS/cm)	n/d	conductivity	n/d		
packing fraction	0.290	packing fraction	n/d		

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Reactor #	67B
Date	October 14, 2004
Inoculum and age	9.0 g FW T16, 14d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	6 days

Comments: roots look brown day 5; very brown (quite DEAD) day 6

Initial:		Final:	
FW (g)	9.0	FW	n/d
DW (g)	0.585	DW	n/d
volume (L)	1.125	volume	0.905
conductivity (mS/cm)	n/d	conductivity	n/d
packing fraction	0.290	packing fraction	n/d

Time (days)	Volume change (mL)
0.8021	-120
3.8854	-180
4.8854	-230
5.8854	-230

Reactor #	68B
Date	October 29, 2004
Inoculum and age	9.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 5% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	6 days

Comments: misting problems every day and cloudy media at harvest

Initial:		Final:			
FW (g)	9.0	FW	12.14	non-blotted bed weight	18.13
DW (g)	0.585	DW	1.1678		
volume (L)	1.125	volume	0.820		
conductivity (mS/cm)	n/d	conductivity	n/d		
packing fraction	0.290	packing fraction	n/d		

Time (days)	Volume change (mL)
1.087	-150
2.917	-190
3.9410	-225
4.7813	-250
5.8750	-260
6.9271	-300

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Reactor #	69B
Date	November 12, 2004
Inoculum and age	9.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 5% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	days

Comments: growth chamber cracked (glued); fast decrease in media level (probably leaked into humidifier base); media and roots brown day 6; misting problems (kinked tubing) day 6

Initial:		Final:	
FW (g)	9.0	FW	n/d
DW (g)	0.585	DW	n/d
volume (L)	1.125	volume	n/d
conductivity (mS/cm)	n/d	conductivity	n/d
packing fraction	0.290	packing fraction	n/d

Time (days)	Volume change (mL)
1.1771	-275
2.8958	-525
3.9583	-600

Reactor #	70A
Date	November 19, 2004
Inoculum and age	9.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 5% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	days

Comments: power outage reset timer (not running) on day 2; misting problems day 3

Initial:		Final:	
FW (g)	9.0	FW	n/d
DW (g)	0.585	DW	n/d
volume (L)	1.125	volume	n/d
conductivity (mS/cm)	n/d	conductivity	n/d
packing fraction	0.290	packing fraction	n/d

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Reactor #	71A
Date	February 18, 2005
Inoculum and age	9.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 6% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	6 days

Comments: no problems; may have been air flow issues on day 2 (stuck flowmeter; changed air pump)

Initial:		Final:			
FW (g)	9.0	FW	10.9	non-blotted bed weight	15.24
DW (g)	0.585	DW	1.1122		
volume (L)	1.125	volume	0.980		
conductivity (mS/cm)	n/d	conductivity	n/d		
packing fraction	0.290	packing fraction	n/d		

Reactor #	72A
Date	February 25, 2005
Inoculum and age	9.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 8% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	4 days

Comments: kinked tubing to mist generator day 4; ended

Initial:		Final:			
FW (g)	9.0	FW	9.96	non-blotted bed weight	13.42
DW (g)	0.585	DW	1.0576		
volume (L)	1.125	volume	0.985		
conductivity (mS/cm)	n/d	conductivity	n/d		
packing fraction	0.290	packing fraction	n/d		

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Reactor #	73A
Date	March 4, 2005
Inoculum and age	9.0 g FW T16, 15d
Culture chamber	50 mL centrifuge tube
Medium	filtered B5, 3% sucrose
Air flow	4 L/min, humidified room air
Misting cycle	15 min on / 15 min off
Culture time	6 days

Comments: timer not working sometime between day 46 (no volume change)

Initial:		Final:			
FW (g)	9.0	FW	10.43	non-blotted bed weight	13.68
DW (g)	0.585	DW	1.0633		
volume (L)	1.125	volume	0.995		
conductivity (mS/cm)	n/d	conductivity	n/d		
packing fraction	0.290	packing fraction	n/d		

Shake flask exp # red dye #1
Date December 19, 2002
Inoculum and age 0.30 g FW T16, 14d
Medium autoclaved B5, 3% sucrose
Culture time 14 days

	FW(g)	DW(g)	vol (L)
Initial	0.30	0.020	0.05
Final			
A	1.626	0.1254	
B	1.600	0.1248	
C	1.770	0.1366	
D	1.487	0.1199	
RA	0.462	0.0452	
RB	0.523	0.0518	
RC	0.446	0.0453	
RD	0.379	0.0394	

key: R = red dye added to media

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Shake flask exp # red dye #2
Date January 17, 2003
Inoculum and age 0.30 g FW T16, 15d
Medium filtered B5, 3% sucrose
Culture time 7 days
Comments: very cold in culture room 2 days before harvest

	FW(g)	DW(g)	vol (L)
Initial	0.30	0.020	0.05
Final			
C1	0.603	0.0549	
C2	0.451	0.0426	
C3	0.596	0.0559	
C4	0.544	0.0499	
R1	0.587	0.0535	
R2	0.578	0.0522	
R3	0.471	0.0436	
R4	0.516	0.0478	
R5	0.630	0.0577	

key: C = control; R = red dye added to media

CONCLUSION: red dye is non-toxic to roots as long as it is not autoclaved

Shake flask exp #	ONE
Date	February 28, 2003
Inoculum and age	0.40 g FW T16, 15d
Medium	filtered B5, 3% sucrose
Culture time	2, 4 days

	FW(g)	DW(g)	vol (L)
Initial	0.40	0.026	0.05
Final			
2A	0.36	0.0315	
2B	0.34	0.0306	
2C	0.34	0.0322	
2D	0.33	0.0308	
2E	0.35	0.0317	
4A	0.44	0.0427	
4B	0.44	0.0399	
4C	0.51	0.0474	
4D	0.45	0.0418	
4E	0.47	0.0465	

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Shake flask exp #	TWO
Date	March 14, 2003
Inoculum and age	0.40 g FW T16, 15d
Medium	filtered B5, 3% sucrose
Culture time	6, 9 days

	FW(g)	DW(g)	vol (L)
Initial	0.40	0.026	0.05
Final			
6A	0.92	0.0843	
6B	0.82	0.0746	
6C	0.96	0.0861	
6D	0.90	0.0843	
6E	0.87	0.0817	
9A	1.02	0.0923	
9B	1.52	0.1255	
9C	1.36	0.1119	
9D	1.55	0.1287	
9E	1.72	0.1382	

Shake flask exp #	THREE
Date	April 11, 2003
Inoculum and age	0.40 g FW T16, 15d
Medium	filtered B5, 5% sucrose
Culture time	2, 4, 6 days

	FW(g)	DW(g)	vol (L)	fructose (g/L)	glucose (g/L)	sucrose (g/L)	gluceqv (g/L)
Initial	0.40	0.026	0.05	0	0	50	52.6316
Final							
2A	0.35	0.0378		2.2893	1.6990	44.4698	50.7987
2B	0.39	0.0403		2.5563	1.9217	44.9126	51.7544
2D	0.36	0.0376		2.5513	1.9546	44.7723	51.6346
4A	0.56	0.0608		5.2824	4.5026	39.9269	51.8134
4B	0.54	0.0582		5.3158	4.5111	39.6198	51.5319
4C	0.55	0.0594		5.2677	4.4056	39.9186	51.6930
6A	0.78	0.0807		8.6428	7.5665	33.6149	51.5934
6B	0.80	0.0862		8.6158	7.4843	33.6013	51.4699
6C	0.76	0.0815		8.5987	7.7355	33.5602	51.6607

	phosphate (g/L)
initial	0.1033
final	
2A	0.0942
2B	0.0932
2C	0.0932
2D	0.0939
4A	0.0807
4B	0.0814
4C	0.0818
6A	0.0708
6B	0.0679
6C	0.0693

Shake flask exp #	FOUR
Date	April 18, 2003
Inoculum and age	0.40 g FW T16, 15d
Medium	filtered B5, 3, 5% sucrose
Culture time	2, 4, 6 days

	FW(g)	DW(g)	vol (L)	fructose (g/L)	glucose (g/L)	sucrose (g/L)	gluceqv (g/L)
Initial	0.40	0.026	0.05	0	0	50	52.6316
Final							
5%							
2A	0.34	0.0360		2.2006	1.7469	45.1696	51.4945
2B	0.38	0.0417		2.3081	1.8380	45.2615	51.7898
2C	0.36	0.0359		2.3944	1.8405	44.9338	51.5337
2D	0.36	0.0407		2.2888	1.7038	45.2614	51.6361
4A	0.48	0.0521		5.4597	4.6776	39.3683	51.5777
4B	0.51	0.0546		5.5238	4.5947	39.6983	51.9061
4C	0.55	0.0575		5.3045	4.3769	40.3728	52.1792
6A	0.72	0.0779		7.7706	6.7116	35.2555	51.5932
6B	0.74	0.0787		8.7005	7.5502	33.5047	51.5188
6C	0.73	0.0798		7.6897	6.5992	35.4268	51.5802
3%							
2A	0.36	0.0333		2.6000	1.9188	25.9456	31.8300
2B	0.37	0.0335		2.5366	1.8426	26.1513	31.9068
2C	0.32	0.0300		2.3574	1.7042	26.1543	31.5924
2D	0.37	0.0338		2.7216	2.0110	25.6976	31.7828
4A	0.55	0.0511		5.7336	4.8351	20.2412	31.8752
4B	0.60	0.0526		5.2619	4.4414	20.6157	31.4040
4C	0.49	0.0479		5.5804	4.5522	20.5497	31.7639
6A	0.94	0.0812		8.8899	7.9326	13.1336	30.6474
6B	0.75	0.0689		9.1208	8.0118	13.3270	31.1610
6C	0.78	0.0710		9.2567	8.0866	13.4668	31.5190

Shake flask exp #		FOUR
Date		April 18, 2003
Inoculum and age		0.40 g FW T16, 15d
Medium		filtered B5, 3, 5% sucrose
Culture time		2, 4, 6 days
phosphate (g/L)		nitrate (g/L)
initial	0.1033	1.5317
final		
5%		
2A	0.0989	1.4457
2B	0.0900	1.4603
2C	0.0939	1.4676
2D	0.0925	1.4384
4A	0.0885	1.4117
4B	0.0861	1.4360
4C	0.0857	1.3800
6A	0.0761	1.3460
6B	0.0761	1.3654
6C	0.0754	1.3119
3%		
2A	0.0935	1.5139
2B	0.0949	1.4871
2C	0.0942	1.5261
2D	0.0949	1.5163
4A	0.0821	1.4117
4B	0.0825	1.4092
4C	0.0836	1.4457
6A	0.0697	1.3654
6B	0.0736	1.3679
6C	0.0732	1.3752

Shake flask exp #	FIVE
Date	July 25, 2003
Inoculum and age	0.40 g FW T16, 15d
Medium	filtered B5, 5% sucrose
Culture time	2, 4, 6, 9, 12 days

	FW(g)	DW(g)	vol (L)	fructose (g/L)	glucose (g/L)	sucrose (g/L)	gluceqv (g/L)
Initial	0.40	0.026	0.05	0	0	50	52.6316
Final							
2A	0.41	0.0372	0.046	2.2053	1.6862	47.1511	53.5242
2B	0.39	0.0354	0.047	2.2818	1.6054	46.8126	53.1636
2C	0.43	0.0370	0.047	2.1892	1.5719	46.7135	52.9331
2D	0.44	0.0364	0.046	2.0742	1.6828	46.9374	53.1648
4A	0.63	0.0581	0.048	4.7180	3.7475	42.0911	52.7719
4B	0.54	0.0511	0.048	4.7888	3.7974	42.4358	53.2554
4C	0.55	0.0524	0.047	4.7183	3.8810	42.0609	52.8739
4D	0.59	0.0525	0.048	5.2101	4.4272	41.2550	53.0636
6A	0.89	0.0849	0.047	8.8742	7.6174	34.1965	52.4880
6B	0.69	0.0640	0.047	7.0321	6.0104	38.2610	53.3172
6C	0.77	0.0720	0.047	9.1330	7.8508	34.2946	53.0834
6D	0.82	0.0752	0.047	7.7302	6.6815	36.8217	53.1713
9A	1.20	0.1191		13.4431	11.6764	25.0032	51.4387
9B	1.00	0.1184		13.9280	12.4072	23.8420	51.4321
9C	1.00	0.1100		13.2329	11.3684	26.5491	52.5478
12A	1.98	0.1783	0.045	18.4156	16.2105	13.9024	49.2602
12B	1.82	0.1630	0.045	19.3039	17.0426	12.8590	49.8824
12D	1.95	0.1728	0.045	19.5694	17.2347	11.5703	48.9834

	phosphate (g/L)	nitrate (g/L)
initial	0.1033	1.5317
final		
2A	0.0921	1.4628
2B	0.0925	1.4701
2C	0.0903	1.4847
2D	0.0900	1.4701
4A	0.0811	1.4555
4B	0.0821	1.4190
4C	0.0804	1.4117
4D	0.0786	1.4238
6A	0.0633	1.3435
6B	0.0740	1.3630
6C	0.0679	1.3557
6D	0.0700	1.3557
9A	0.0480	1.1853
9B	0.0487	1.2170
9C	0.0519	1.1975
12A	0.0263	1.0125
12B	0.0260	1.0539
12C	0.0441	1.1439
12D	0.0196	0.9711

Shake flask exp #	SIX
Date	August 1, 2003
Inoculum and age	0.40 g FW T16, 15d
Medium	filtered B5, 6, 8, 10% sucrose
Culture time	14 days

	FW(g)	DW(g)	vol (L)	fructose (g/L)	glucose (g/L)	sucrose (g/L)	gluceqv (g/L)
Initial							
6	0.40	0.026	0.05	0	0	60	63.1579
8	0.40	0.026	0.05	0	0	80	84.2105
10	0.40	0.026	0.05		0	100	105.2632
Final							
6A	2.01	0.1956	0.044	21.7015	19.4315	15.9222	57.8932
6B	1.89	0.1848	0.045	23.8893	21.3092	13.3432	59.2439
6C	1.61	0.1566	0.045	19.7044	17.4573	20.3705	58.6043
6D	2.01	0.1879	0.043	20.1048	17.3380	19.5505	58.0222
8A	1.61	0.1586	0.048	20.8219	18.7123	38.0313	79.5672
8B	1.61	0.1647	0.043	22.6683	19.3919	36.9219	80.9254
8C	1.38	0.1423	0.045	23.4038	19.7355	36.6300	81.6972
8D	1.38	0.1417	0.046	23.8860	19.9456	35.8755	81.5953
10A	1.08	0.1133	0.046	21.9876	17.8057	59.0965	102.0002
10B	1.16	0.1180	0.046	21.6905	17.4125	59.9184	102.1750
10C	1.20	0.1243	0.045	23.2555	18.9668	57.3622	102.6036
10D	1.04	0.1094	0.047	21.9340	17.3718	61.0312	103.5492

	phosphate (g/L)	nitrate (g/L)
initial	0.1033	1.5317
final		
6A	0.0192	0.9529
6B	0.0196	1.0727
6C	0.0352	1.1802
6D	0.0210	1.0409
8A	0.0373	1.1704
8B	0.0299	1.0507
8C	0.0363	1.1411
8D	0.0377	1.1484
10A	0.0469	1.2266
10B	0.0473	1.1729
10C	0.0423	1.1900
10D	0.0508	1.1875

Shake flask exp #	SEVEN
Date	August 8, 2003
Inoculum and age	0.40 g FW T16, 15d
Medium	filtered B5, 3% sucrose
Culture time	2, 4, 6 days

	FW(g)	DW(g)	vol (L)	fructose (g/L)	glucose (g/L)	sucrose (g/L)	gluceqv (g/L)
Initial	0.40	0.026	0.05	0	0	30	31.5789
Final							
2A	0.39	0.0338	0.048	2.5578	1.9023	26.0615	31.8932
2B	0.38	0.0325	0.048	2.5591	1.8038	26.2595	32.0045
2C	0.38	0.0381	0.048	2.5008	1.7816	26.0915	31.7471
4A	0.55	0.0492	0.048	5.3638	4.5896	20.7489	31.7944
4B	0.56	0.0473	0.047	5.7470	5.0121	21.3841	33.2688
4C	0.52	0.0462	0.048	5.4653	4.4493	20.8524	31.8645
6A	0.72	0.0604	0.048	5.6336	7.3261	14.5655	28.2918
6B	0.71	0.0572	0.047	8.0613	6.9102	15.3918	31.1733
6C	0.81	0.0633	0.048	8.0783	7.1719	14.6658	30.6879
6D	0.72	0.0571	0.047	7.8093	6.6892	15.7087	31.0340
9A	1.24	0.0977	0.046	13.6482	11.005	5.75554	30.7115
9B	1.15	0.0909	0.046	6.84592	6.1741	3.43268	16.6334
9C	1.25	0.1005	0.045	14.6592	12.0088	3.52968	30.3835
12A	1.78	0.1369	0.045	15.6003	12.9394	0	28.5397
12B	1.77	0.1328	0.045	15.5376	12.8552	0	28.3928
12C	1.90	0.1379	0.045	15.2765	12.5575	0	27.8340
12D	1.64	0.1232	0.045	15.2007	12.8449	0	28.0456

	phosphate (g/L)	nitrate (g/L)
initial	0.1033	1.5317
final		
2A	0.0971	1.4969
2B	0.0953	1.4944
2C	0.0942	1.4530
4A	0.0836	1.4141
4B	0.0825	1.4117
4C	0.0857	1.4774
6A	0.0708	1.4530
6B	0.0697	1.4311
6C	0.0743	1.4433
6D	0.0729	1.2754
9A	0.0562	1.2510
9B	0.0405	1.1804
9C	0.0512	1.2583
12A	0.0306	1.1902
12B	0.0295	1.1585
12C	0.0281	1.1147
12D	0.0370	1.1634

Shake flask exp #	EIGHT
Date	August 22, 2003
Inoculum and age	0.40 g FW T16, 15d
Medium	filtered B5, 1.8, 3, 5% sucrose
Culture time	14 days

	FW(g)	DW(g)	vol (L)	fructose (g/L)	glucose (g/L)	sucrose (g/L)	gluceqv (g/L)
Initial							
1.8	0.40	0.026	0.05	0	0	18	18.9474
3	0.40	0.026	0.05	0	0	30	31.5789
5	0.40	0.026	0.05	0	0	50	52.6316
Final							
1.8A	1.56	0.0946	0.045	12.2051	11.9239	0	24.1290
1.8B	1.56	0.1025	0.045	9.0863	6.2678	0	15.3542
1.8C	1.65	0.1097	0.042	9.4665	6.4641	0	15.9307
1.8D	1.44	0.0918	0.045	7.1548	5.3160	0	12.4708
1.8E	1.75	0.1121	0.045	9.5197	6.5529	0	16.0725
3F	1.81	0.1328	0.045	15.5617	12.5549	0	28.1156
3G	1.99	0.1453	0.045	15.3958	11.8283	0	27.2241
5H	1.72	0.1643	0.045	23.7799	21.0082	2.7083	47.6389
5I	1.95	0.1817	0.045	24.1479	20.6688	3.5261	48.5284
5J	1.84	0.1656	0.045	23.9606	20.5672	4.4976	49.2621

	phosphate (g/L)	nitrate (g/L)
initial	0.1033	1.5317
final		
1.8A	0.0565	1.3219
1.8B	0.0473	1.3341
1.8C	0.0377	1.2584
1.8D	0.0398	0.9872
1.8E	0.0412	1.2388
3F	0.0245	1.2242
3G	0.0160	1.1606
5H	0.0224	1.1264
5I	0.0153	1.0531
5J	0.0203	1.1240

Shake flask exp #	NINE
Date	December 2003
Inoculum and age	0.50 g FW T16, 15d
Medium	filtered B5, 3% sucrose, glucose, or fructose
Culture time	14 days

	FW(g)	DW(g)	vol (L)	fructose (g/L)	glucose(g/L)	sucrose (g/L)	gluceqv (g/L)
Initial							
F	0.50	0.0325	0.05	31.578	0	0	31.5789
G	0.50	0.0325	0.05	0	31.5789	0	31.5789
S	0.50	0.0325	0.05	0	0	30	31.5789
Final							
F1	1.78	0.1271	0.044	29.4162	0	0	29.4162
F2	2.13	0.1884	0.043	25.3789	0	0	25.3789
F3	2.29	0.2045	0.042	24.6834	0	0	24.6834
F4	2.51	0.1919	0.041	25.5339	0	0	25.5339
F5	2.43	0.2125	0.042	24.4554	0	0	24.4554
G1	1.17	0.0819	0.045	0	27.4797	0	27.4797
G2	0.71	0.0605	0.047	0	28.5264	0	28.5264
G3	1.55	0.1189	0.044	0	26.4007	0	26.4007
G4	1.49	0.1162	0.043	0	26.6635	0	26.6635
G5	1.44	0.1152	0.045	0	26.5955	0	26.5955
S1	2.37	0.1659	0.042	15.7000	10.9757	0	26.6757
S2	2.57	0.1758	0.043	15.2982	10.9480	0	26.2462
S3	2.63	0.1891	0.041	16.2468	11.1375	0	27.3843
S4	3.02	0.2078	0.042	16.0647	10.9995	0	27.0642
S5	2.50	0.1980	0.044	15.9761	11.0071	0	26.9832

	phosphate (g/L)	nitrate (g/L)
initial	0.1033	1.5317
final		
F1	0.0260	1.2193
F2	0.0096	1.1460
F3	0.0021	0.9065
F4	0.0039	1.0018
F5	0.0039	1.1142
G1	0.0555	1.4245
G2	0.0658	1.4929
G3	0.0249	1.3024
G4	0.0306	1.3317
G5	0.0309	1.3317
S1	0.0075	1.0409
S2	0.0046	1.0629
S3	0.0039	1.0482
S4	0.0018	0.8772
S5	0.0025	1.0727

Shake flask exp #	TEN
Date	April 30, 2004
Inoculum and age	0.40 g FW T16, 15d
Medium	filtered B5, 3% sucrose
Culture time	14, 21, 28 days

	FW(g)	DW(g)	vol (L)	fructose (g/L)	glucose (g/L)	sucrose (g/L)	gluceqv (g/L)
Initial	0.40	0.026	0.05	0	0	30	31.5789
Final							
1A	2.05	0.1517					
1B	2.09	0.1578					
1C	1.93	0.1417					
1D	2.35	0.1618					
2A	2.56	0.1785					
2B	2.28	0.1664					
2C	2.14	0.1585					
2D	2.50	0.1703					
1rA	1.33	0.1150	0.039	1.3798	0.8429	0	22.2271
1rB	1.18	0.1060	0.038	1.3850	0.8212	0	22.0625
1rC	1.19	0.1100	0.040	1.4686	0.9003	0	23.6888
1rD	1.09	0.0940	0.040	1.4397	0.8432	0	22.8288
2rA	1.19	0.1080	0.039	1.4182	0.8343	0	22.5251
2rB	1.33	0.1170	0.039	1.6207	0.9131	0	25.3378
2rC	1.43	0.1170	0.040	1.4283	0.8327	0	22.6096
2rD	1.26	0.1200	0.038	1.5489	0.7968	0	23.4571
3A			0.042	1.5419	1.1745	0	27.1634
3B			0.043	1.5697	1.2001	0	27.6974
3C			0.043	1.5211	1.2685	0	27.8959
3D			0.043	1.5380	1.2121	0	27.5011
3B-21	4.97	0.3670	0.0455	1.5466	1.0421	0	25.8870
3C-21	4.91	0.3640	0.043	1.0565	0.8094	0	18.6585
3D-21	4.78	0.3560	0.0435	1.4941	1.1108	0	26.0490
4A	4.42	0.3030	0.038	1.4759	0.7754	0	22.5136
4B	4.57	0.3430	0.037	1.4033	0.6304	0	20.3371
4C	4.58	0.3440	0.038	1.3824	0.6208	0	20.0315
4D	4.94	0.3320	0.0355	1.4284	0.5835	0	20.1192

key: 1 = remove roots @14d, reinoculate with new roots and grow for 14 more days (1r); 2 = remove roots @14d, filter media, reinoculate with new roots and grow for 14 more days (2r); 3 = drain media @14d, add fresh media and grow for 7 more days (3x21); 4 = 21 d control

Shake flask exp #	ELEVEN
Date	June 25, 2004
Inoculum and age	0.40 g FW T16, 15d
Medium	filtered B5, 5, 10% sucrose or mannitol
Culture time	14 days

	FW(g)	DW(g)	vol (L)	fructose (g/L)	glucose (g/L)	sucrose (g/L)	gluceqv (g/L)
Initial							
A	0.40	0.026	0.05	0	0	100	105.2632
B	0.40	0.026	0.05	0	0	50	52.6316
C	0.40	0.026	0.05	0	0	50	52.6316
D	0.40	0.026	0.05	0	0	0	0
Final							
A1	1.13	0.1356	0.047	18.5800	17.5876	6.2894	102.3719
A2	1.05	0.1297	0.047	20.5828	19.9181	6.0661	104.3543
A3	1.01	0.1280	0.046	23.6227	21.6058	5.2223	100.1996
A4	0.97	0.1189	0.047	29.7294	28.5352	6.1313	122.8043
A5	1.04	0.1301	0.044	22.3530	21.2296	6.1256	108.0625
B1	1.13	0.1302	0.046	18.2973	16.1746	1.5430	50.71440
B2	1.16	0.1279	0.046	18.7963	17.2695	1.3158	49.91627
B3	1.11	0.1287	0.046	24.1998	22.2800	0.6721	53.55455
B4	1.08	0.1157	0.047	20.0712	18.1497	1.0315	49.07827
B5	1.07	0.1224	0.0475	22.8883	20.8916	0.6152	50.25619
D1	1.48	0.1595	0.047	23.8450	21.4350	0.2401	47.80747
D2	1.60	0.1687	0.046	23.6919	21.2551	0.5405	50.63673
D3	1.53	0.1596	0.046	23.7614	21.2373	0.6349	51.68172
D4	1.62	0.1673	0.046	24.5619	22.3238	0.3795	50.88056
D5	1.56	0.1581	0.046	26.2510	23.3977	0.0994	50.69540
C1	0.36	0.0238	0.048				
C2	0.37	0.0227	0.048				
C3	0.37	0.0249	0.048				
C4	0.38	0.0240	0.048				
C5	0.37	0.0246	0.046				

key: A = 10% sucrose; B = 5% sucrose + mannitol (osmotically equivalent to 10% sucrose); D = 5% sucrose; C = mannitol

Shake flask exp #	TWELVE		
Date	September 10, 2004		
Inoculum and age	0.40 g FW T16, 15d		
Medium	filtered B5, 3, 5, 6, 8, 10% sucrose or glucose/fructose ratio=0.85		
Culture time	14 days		
	FW(g)	DW(g)	vol (L)
Initial	0.40	0.026	0.05
Final			
3SA	2.11	0.1514	0.0455
3SB	1.62	0.1198	0.0465
3SC	1.94	0.1382	0.046
5SA	1.30	0.1232	0.047
5SB	1.60	0.1511	0.0465
5SC	1.53	0.1435	0.047
5SD	1.43	0.1340	0.0475
6SA	1.54	0.1528	0.0465
6SB	1.48	0.1473	0.0465
6SC	1.42	0.1365	0.0465
6SD	1.53	0.1511	0.0465
8SA	1.19	0.1256	0.047
8SB	1.26	0.1299	0.047
8SC	1.38	0.1428	0.046
8SD	1.29	0.1361	0.0465
10SA	0.88	0.1024	0.0475
10SB	0.82	0.0944	0.0475
10SC	1.07	0.1208	0.047
3A	0.90	0.0693	0.0465
3B	1.00	0.0787	0.047
3C	1.11	0.0845	0.047
3D	0.76	0.0632	0.0465
5A	0.76	0.0707	0.0475
5B	0.99	0.0919	0.0475
5C	0.91	0.0857	0.048
5D	0.91	0.0842	0.0485
6A	0.73	0.0781	0.0475
6B	0.53	0.0553	0.048
6C	0.76	0.0759	0.0475
6D	0.83	0.0790	0.0475
8A	0.53	0.0666	0.0475
8B	0.48	0.0606	0.048
8C	0.55	0.0663	0.0485
8D	0.58	0.0691	0.0445
10A	0.28	0.0397	0.0485
10B	0.34	0.0462	0.0485
10C	0.39	0.0539	0.048
10D	0.38	0.0523	0.0485

key: S = sucrose; others are G/F ratio = 0.85

Shake flask exp #	THIRTEEN
Date	October 8, 2004
Inoculum and age	0.40 g FW T16, 15d
Medium	filtered B5, 3% sucrose
Culture time	7, 14, 28 days

	FW(g)	DW(g)	vol (L)	fructose (g/L)	glucose (g/L)	sucrose (g/L)	gluceqv (g/L)
Initial	0.40	0.026	0.05	0	0	30	31.5789
Final							
7A	0.72	0.0619	0.048				
7B	0.76	0.0621	0.048				
7C	0.86	0.0752	0.048				
7D	0.84	0.0698	0.048				
14A			0.044				
14B			0.047				
14C			0.046				
14D			0.046				
14E			0.0465				
14-28A	8.32	0.6428	0.032				
14-28B	6.48	0.4945	0.036				
14-28C	7.83	0.5767	0.033				
14-28D	8.75	0.5917	0.033				
14-28E	6.71	0.4904	0.035				
28A	4.06	0.3618	0.040				
28B	3.47	0.3288	0.041				
28C	5.05	0.4241	0.038				
28D	4.90	0.4243	0.038				
28E	4.00	0.3509	0.041				

key: 7 = 7 d control; 14=14 d media from 14-28 cultures; 14-28 = fresh media @14d and grown for 14 more days; 28 = 28 d control

Shake flask exp #	ALPHA
Date	October 21, 2004
Inoculum and age	14.5 g FW T16, 14d
Medium	filtered B5, 3% sucrose
Culture time	6 days

	FW(g)	DW(g)	vol (L)	fructose (g/L)	glucose (g/L)	sucrose (g/L)	gluceqv (g/L)
Initial	14.5	0.9425	0.05	0	0	30	31.5789
Final	12.82	0.9681	0.033				

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Shake flask exp #	FOURTEEN
Date	December 3, 2004
Inoculum and age	0.40 g FW T16, 15d
Medium	filtered B5, 5, 7% sucrose or mannitd
Culture time	14 days

	FW(g)	DW(g)	vol (L)	fructose (g/L)	glucose (g/L)	sucrose (g/L)	gluceqv (g/L)
Initial							
5	0.40	0.026	0.05	0	0	100	52.6316
5M	0.40	0.026	0.05	0	0	50	52.6316
7	0.40	0.026	0.05	0	0	50	73.6842
Final							
5A	1.51	0.1499					
5B	1.28	0.1276					
5C	1.17	0.1158					
5D	1.28	0.1289					
5MA	0.86	n/d					
5MB	1.00	n/d					
5MC	0.91	n/d					
7A	1.15	0.1195					
7B	1.09	0.1089					
7C	0.98	0.1005					
7D	1.08	0.1121					

key: 5 = 5% sucrose; 5M = 5% sucrose + mannitol (osmotically equivalent to 7% sucrose); 7 = 7% sucrose