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Mist reactors: principles, comparison of various systems, and case studies

Pamela Weathers[1,2], Chunzhao Liu[1,3], Melissa Towler[2], and Barbara Wyslouzil[4]

[1] Arkansas State University, Jonesboro, AR; [2] Worcester Polytechnic Institute, Worcester, MA;
[3] National Key Laboratory of Biochemical Engineering, Beijing, China; [4] The Ohio State University, Columbus, OH
*Corresponding author; email: weathers@wpi.edu

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ABSTRACT

Growing hairy roots in bioreactors has proven challenging. Here we summarize the recent work using a novel bioreactor, the mist reactor, for the culture of hairy roots from many plant species. Compared to most liquid-phase bioreactors, this gas-phase bioreactor offers a completely different environment for growing roots. Design, modeling, and some of the unique engineering aspects inherent to mist reactors are explained along with some of the biological responses of roots when grown in mists compared to liquids. Prospects for future development of this technology are also summarized.

INTRODUCTION

Hairy roots can produce novel compounds not found in the whole plant or in untransformed roots (Flores and Medina-Bolivar, 1995; Wysokinska and Chmiel, 1997; Flores et al., 1998), often at levels exceeding those found in the parent plant (Wysokinska and Chmiel, 1997; Wysokinska and Rozga, 1998; Rao and Ravishankar, 2002). They also offer significant advantages for the production of engineered proteins (Guillon et al., 2006; Zhang et al., 2005; Shanks and Morgan, 1999). Although there are some reports of co-cultured differentiated tissues (e.g. shoots plus roots) being used to produce secondary metabolites (Mahagamasekera and Doran, 1998; Subroto et al., 1996), most efforts are focused on hairy roots.

Several bottlenecks, however, hamper the routine production of compounds, especially secondary metabolites, from hairy roots. The two main challenges are: (1) for many products the biochemical pathways and their regulation are not understood; and (2) although progress has been made, cost effective scaleable production systems are not yet well developed. Developing an inexpensive scaleable reactor for hairy roots would address the latter problem, enabling large-scale culture and the production of many important and valuable chemicals (Guillon et al., 2006). Recent political challenges to the release of transgenic plants further warrant the use of controlled production schemes including the use of low cost bioreactors (Guillon et al., 2006).

Our research has focused on gas-phase (mist) reactors, because this environment reduces the gas-exchange limitations and the high shear conditions normally found in liquid-phase reactors. Unlike growth in liquid systems, roots grown in mist reactors are not oxygen limited (Weathers et al., 1999) even at high bed densities (Kim, 2001), and the production of secondary metabolites is often greater in mist reactors than in liquid phase reactors (Kim et al., 2001; Bais et al., 2002). High biomass density is required for a reactor system to be economically viable, and the maximum root tissue concentration that can be achieved in a bioreactor depends on the efficient mass transfer of oxygen and other nutrients into the dense root matrix (Curtis, 2000). A point in favor of gas phase reactors is that roots are more able to compensate for poor liquid dispersion than for poor gas dispersion within a reactor (McKelvey et al., 1993). A recent comparison by Suresh et al. (2005), also verified that a mist bioreactor is superior to other reactor designs for hairy root growth. Finally, establishing a hairy root culture on solid medium does not guarantee that they will grow well in liquid medium (Hallard et al., 1997), so gas-phase reactors may be the only option. Together these results provide compelling reasons to study mist reactors.

Recent reviews have added new perspectives regarding the prospects and challenges of producing secondary metabolites from hairy roots in bioreactors (Kim et al., 2002; Towler et al., 2006; Weathers et al., 2006). These reactors, however, have not yet been scaled to commercially useful sizes. The key scale-up concerns for hairy root bioreactors include the following: the flow and geometric configurations should be scale independent; high growth rates must be maintained with no limitations in liquid and gas (O_2) nutrients; the key operating parameters must be identifiable and measurable so that scale-up can be achieved easily and economically (Cuello et al., 2003). Here we provide a review on mist/spray reactors and the efforts to scale them up.

LIQUID VS. GAS PHASE REACTORS

O_2, CO_2 and C_2H_4 are the three gases most important to plants, but all are poorly soluble in water; their solubility decreases with increasing nutrient concentration and temperature (Atkinson and Mavituna, 1991; Geankopolis, 1993), and oxygen is about 25 times less soluble than CO_2. To ensure rapid growth of roots, high levels of oxygen are required while excessive CO_2 accumulation must be prevented as the level of respiration increases with increasing root biomass. Liquids therefore limit the amount of gas available to growing roots.

Good gas transfer into liquid media can be obtained by either dispersing the liquid into the gas or by dispersing the gas into the liquid. In liquid-based reactors, gas is dispersed into the liquid phase, while in a mist reactor liquid is dispersed into the gas phase. Liquid dispersion can be achieved using a number of different methods including a spinning disk, a compressed gas atomizer, or an ultrasonic droplet generator. The spinning disk produces a wide range of droplet sizes and is mainly used in whole plant aeroponic systems (Weathers and Zobel, 1992). Smaller droplets have a larger surface to volume ratio that facilitates gas transport into liquid. Compared to the spinning disk, compressed gas atomizers can produce rather uniform amounts of very small droplets thereby ensuring good gas transfer into the liquid and thus, to the roots. Unfortunately to obtain small droplets, the nozzle orifice must be small and, as a result, it clogs easily.

Ultrasonically produced mists have proved to be the most effective method of generating nutrient aerosols for *in vitro* cultures and have, thus, been the focus of most mist reactor studies with hairy roots. Earlier mist reactors used a submerged ultrasonic transducer producing sound waves in the 1.7 MHz range. Although the mechanism of energy transmission of this type of misting system has been discussed in depth elsewhere (Weathers and Wyslouzil, 2007), the transducer energy can affect the mean droplet diameter, D_n, which depends on the density ρ the surface tension of the liquid σ and the transducer frequency ν as $D_n = 0.34(8\pi\sigma/\rho\ \nu^2)^{(1/3)}$ (Lang, 1962). At higher frequencies D_n also depends on the power density at the liquid surface (Tarr et al., 1991).

One of the most important factors in a mist or spray reactor is the droplet size, and Table 1 summarizes the three broad categories generally used to classify droplets dispersed in a gas. Smaller droplets have several advantages including long settling times that ease transport and distribution of the dispersed nutrient solution and enhance the ability of the small droplets to penetrate dense tissue cultures and more evenly distributing liquid nutrients, as well as large surface to volume ratios that ensure good mass transfer of gases between the gas and liquid phases enhancing gas transfer to the root tissue. If the droplet size is too small, however, inadequate medium will be supplied to the roots. Excessively large droplets, on the other hand, will impede adequate gas transfer into the roots. Although the optimum droplet size is not known, and has not been studied other than by measuring droplet deposition in dense beds of hairy roots (Wyslouzil et al., 1997), Weathers and Zobel (1992) suggested that, for roots, the minimum acceptable droplet size is probably about 1 μm.

Table 1. Droplet size ranges for mists, fogs and sprays. (Perry and Green, 1997; Weathers and Zobel, 1992; Whipple, 1995).

Droplet type	Droplet diameter (μm)	Droplet forming system	Droplet diameter (μm)
Mist	0.01-10	ultrasonic systems	1-35 Dv ~ 7-10 is typical
Fog	1-100	compressed gas atomizers	1-100
Spray	10-10,000	spray nozzles	>100

A BASIC MIST REACTOR

A basic mist bioreactor (Figure 1) is rather simple. It is comprised of a mist generating system, a culture medium reservoir, a liquid pump (usually peristaltic), a sterile filtered air supply, a gas flow meter, a timer to regulate misting time, and a culture chamber. Most mist reactors also require a trellis or other means of support for suspending roots in the growth chamber.

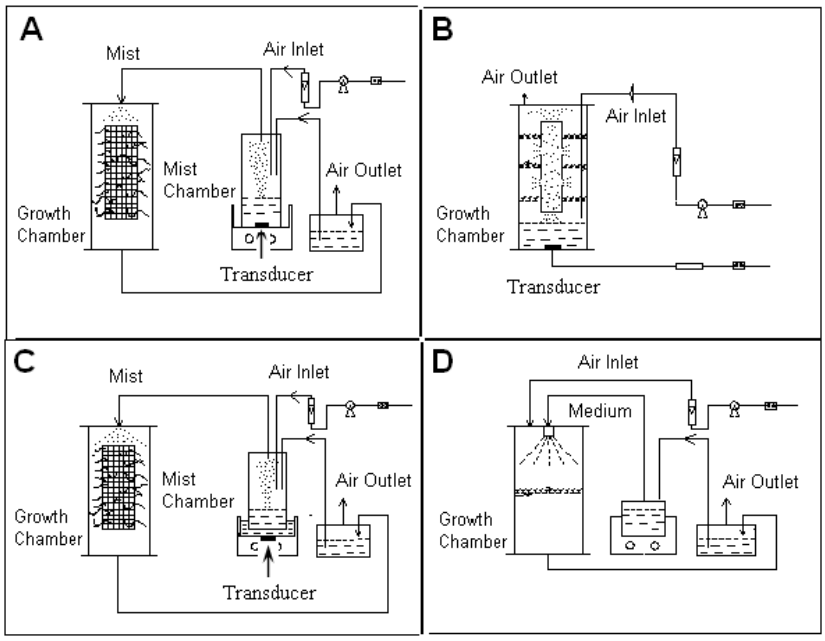

Figure. 1 Mist bioreactor designs for hairy root cultures: A) the ultrasonic mist generator and growth chamber are in separate vessels; the ultrasonic transducer (arrow) is inside the mist generator sump; B) the ultrasonic mist generator

Special Issue on Hairy Roots (A. Lorence and F. Medina-Bolivar, co-editors)

and the growth chamber are in the same vessel; C) the ultrasonic transducer (arrow) is separated from the growth chamber sump by an acoustic window which is also the base of the mist generator; D) trickle bed bioreactor.

Table 2. Summary of hairy root studies in mist or spray reactors.

Plant Genus	Major Results	System Used	Working Vol. (L)	Reference
Artemisia	growth comparable to flasks and plates	acoustic window[1] mist reactor	na	Buer et al., 1996
	modified inner-loop reactor growth comparable to flasks	submerged ultrasonics	2.3	Liu et al., 1999
	no O_2 limitation, but 50% less biomass than liquid systems	acoustic window[2] mist reactor	1.5	Weathers et al., 1999
	altered branching rate versus flasks – single root studies	acoustic window[2] mist reactor	na	Wyslouzil et al., 2000
	3x higher artemisinin content than bubble column	acoustic window[2] mist reactor	1.5	Kim et al., 2001
	growth comparable to bubble column	acoustic window[2] mist reactor	1.5	Kim et al., 2002a
	altered terpenoid gene expression versus flasks	acoustic window[2] mist reactor	1.5	Souret et al., 2003
	in 5% sucrose growth exceeded that in shake flasks	acoustic window[2] small mist reactor	0.035	Towler et al., 2007
Beta	growth comparable to flasks	submerged ultrasonics	1	Weathers et al., 1989
Carthamus	growth comparable to flasks; 15% faster than airlift reactor	submerged ultrasonics	1	DiIorio et al., 1992
Cichorium	higher biomass and esculin content than bubble column	acoustic window[3] mist reactor	1.75	Bais et al., 2002
Datura	1.6x lower doubling time than submerged cultures	droplet reactor	2	Wilson et al., 1990
	successful large-scale culture	hybrid submerged/ droplet reactor	500	Wilson, 1997
Fragaria	biomass yield higher than droplet bioreactor	mist reactor	1	Nuutila et al., 1997
Hyoscyamus	growth comparable to shake flask	spray reactor	0.30	McKelvey et al., 1993
Nicotiana	50% lower doubling time than flasks	spray reactor	0.50	Whitney, 1990, 1992
Panax	altered ginsenoside pattern versus native rhizome	spray reactor	1	Palazon et al., 2003
Stizolobium	intermittent feeding promoted L-DOPA production	mist trickling reactor	3	Huang et al., 2004
	higher L-DOPA productivity with higher inoculum density	mist trickling reactor	9	Huang and Chou, 2006
	root branching increased biomass and L-DOPA content	mesh hindrance mist trickling reactor	5	Sung and Huang, 2005
Tagetes	growth and thiophene content comparable to shake flask; higher than bubble column, nutrient sprinkle	acoustic window[3] mist reactor	0.30	Suresh et al., 2005

1, Conap's EN6, 2, Teflon, 3, polycarbonate, na, not applicable.

It is possible that the size of such a support structure can be minimized if it is coated with poly-L-lysine to induce root attachment (Towler et al., 2003). How these components are designed and then configured has enabled a variety of mist reactors to be employed for culturing many different biological tissues or organisms. For example, mist reactors have been successfully used to culture animal tissues (Friberg et al., 1992), to study whole plants (Souret and Weathers, 2000; Sharaf-Eldin and Weathers, 2006), in micropropagation (Correll and Weathers, 2001a, 2001b; Correll et al., 2001; Weathers and Giles, 1988 and 1989), and to culture hairy roots (Table 2). Figure 1 illustrates some mist reactor designs that have been employed for hairy root culture.

NUTRIENT MIST DROPLET DEPOSITION MODEL

In experiments by Kim et al. (2002), roots grown in a bubble column reactor consistently yielded more dry mass than roots harvested from a mist reactor. After comparing the biological demand of the growing roots in the mist reactor to the level of substrate (sugar) deposited on the roots as a function of reactor packing fraction, and the deposition of medium droplets using a mist deposition model (Wyslouzil et al., 1997), Kim et al. (2002) suggested that roots at low packing fraction could not capture enough mist to grow at rates comparable to roots grown in liquid culture. At higher packing fractions, on the other hand, the opposite appeared to be true – roots grew more rapidly.

In the mist deposition model (Wyslouzil et al., 1997), root beds are treated as if they are fibrous filters. For a fixed droplet size D_p, the droplet capture efficiency (η_B) of the root bed is given by η_B = 1-exp[(-4$L\alpha\eta_C$) / (D_R(1- α))], where α is the packing fraction, L is the length of the root bed, D_R is the diameter of the root and η_C is the combined capture efficiency due to impaction and interception ($\eta_{IMP+INT}$), and diffusion (η_D), and these efficiencies are all functions of D_p (Crawford, 1976; 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: $\eta_{OM} = \sum_i \eta_B(D_{Pi}) \times m(D_{Pi})$. Finally, the medium captured by the roots (V_{dep}, mL d^{-1}) is expressed as: $V_{dep} = 24\omega \times Q_L \times \eta_{OM}$, where the factor of 24 converts from hr to d, ω is the duty cycle (min hr^{-1}), and Q_L is the medium flow rate (mL min^{-1}) during the mist "on" cycle. The model is valid only when the flow rate is low and the Reynold's number (Re), based on the root diameter, is < 10 (Wyslouzil et al., 1997).

The medium required to support the growth of roots (V_{req},, mL d^{-1}), in turn, depends on the amount of biomass present, its growth rate μ (d^{-1}), the apparent biomass yield of the growth-limiting nutrient $Y_{X/S}$ (g DW biomass per g nutrient consumed) and the concentration of the limiting nutrient in the medium C_S (g L^{-1}). To maintain a desired growth rate, μ, $V_{dep} \geq V_{req}$. Kim et al. (2002) suggested that at low packing fractions a lack of nutrients was likely limiting *A. annua* hairy root growth in the mist bioreactor. Since V_{dep} is a strong non-linear function of α, increasing α rapidly increases V_{dep} thereby supporting a higher growth rate because the roots are now capturing more nutrients. Although V_{req} also depends on α, that relationship is linear. Kim et al. (2002) showed that V_{dep} can be greater than V_{req}, but depends on the nutrient concentration, as well as the growth rate and diameter of the roots.

LAB SCALE STUDIES

Exploiting the Mist Deposition Model to Improve Root Growth in Mist Reactors

The mist deposition model suggested several ways to improve the growth rate of roots in a mist reactor beyond that observed by Kim et al. (2002). First, increasing the initial biomass density (packing fraction, α) should increase the droplet capture efficiency of the roots and better satisfy the biological demand. Second, increasing the total carbon available to the roots, either by increasing the sugar concentration (CS) of the medium or by increasing the mist duty cycle (ω), should also increase the growth rate. To test these hypotheses, Towler et al. (2007) constructed a small mist reactor (0.05 L growth chamber) and conducted an extensive number of short (6 day) root growth experiments.

As illustrated in Figure 2, the measured growth rates of 0.12 d^{-1} over a 6-day period at initial packing fractions in the small mist reactor ranging from 0.19-0.39. In the work of Kim et al. (2002), packing fractions were no more than 0.056 when mist mode commenced and the growth rate (μ) was ~ 0.07 d^{-1} (points at α <0.1 in Figure 2). The work by Towler et al. (2007), confirmed that a higher initial inoculum density does positively affect root growth in mist reactors.

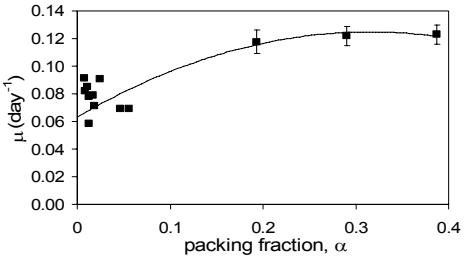

Figure 2. Hairy root growth vs. packing fraction, α, in mist reactors.

To test the limits of packing density that can be achieved in a mist reactor, roots were grown in the small mist reactor from an initial α of 0.39 for 10.3 days. Although the average growth rate was only 0.11 d^{-1}, the final α was 0.71, and, as illustrated in Figure 3, the harvested roots appeared non-necrotic and healthy throughout the dense bed. In liquid-phase culture such high biomass densities are difficult to achieve because at tissue concentrations approaching 10-40 g DW L^{-1} (a range of α ~ 0.20-0.80) the root bed resists fluid flow resulting in too high a pressure drop (Carvalho and Curtis, 1998; Ramakrishnan and Curtis, 2004) with a liquid flow resistance similar to a column of packed sand (Carvalho and Curtis, 1998; Nield and Bejan, 1991).

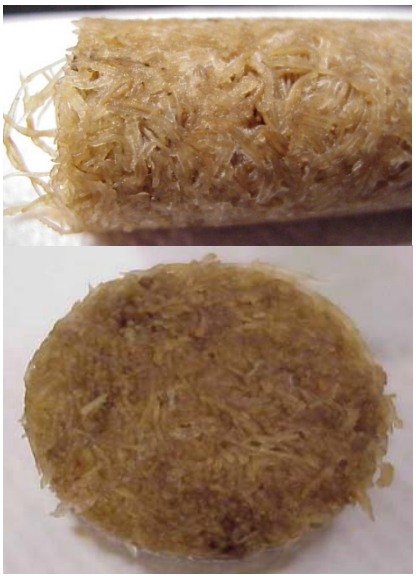

Figure 3. Roots removed from small mist reactor. Final packing density = 0.71; root bed diameter is 2.7 cm. Upper panel is side view; lower panel is cross sectional view of roots half way down the height of the bed.

Besides the packing density, α, one of the other key components of the mist deposition model is the nutrient concentration, C_S. To grow at a particular growth rate, μ, the volume of medium required by the roots, V_{req}, is inversely proportional to C_S. Increasing C_S should, therefore, increase μ at fixed V_{dep}, or decrease V_{req} at fixed μ. Towler et al. (2007) tested this hypothesis using the small (0.05 L) mist reactor. *A. annua* hairy roots were fed medium containing 3% or 5% sucrose at a standard mist duty cycle (15 min on, 15 min off). After 6 days, roots grown with 5% sucrose had significantly more biomass

than roots grown with 3% sucrose (Table 3), while the highest growth rate occurred at day 4. The growth rate at day 4 with 5% sucrose in the mist reactor is equivalent to that in shake flasks. Although we have tried to grow roots in shake flasks at α values equivalent to what we used in the small mist reactor, there was little to no growth (Towler, 2005). Taken together these results corroborate the mist deposition model's (Wyslouzil et al., 1997) prediction that at lower root bed packing densities (α ≤ 0.1) roots cannot capture all of the droplets. Thus, especially in the early stages of operation (just after inoculation), media with a higher sucrose concentration must be provided to deliver adequate levels of carbon to achieve and sustain a high growth. Although most of the droplets are captured once α reaches ~ 0.2, maintaining high growth rates in these densebeds also requires maintaining a high sucrose concentration to feed the dense root population.

Table 3. Sugar concentration increases growth in mist bioreactors after 4 and 6 days. Initial DW of inoculum was 8 g FW L^{-1} (0.52 g DW L^{-1}).

Growth Condition (working volume)	DW (g)		μ (d^{-1})	
Mist reactor (35 mL)	4 d	6 d	4 d	6 d
3% sucrose	1.11	1.22	0.16	0.12
5% sucrose	1.29	1.77	0.20	0.13
Shake flasks (50 mL)				
3% sucrose	0.05	0.07	0.15	0.17
5% sucrose	0.06	0.08	0.19	0.18

An alternate method for providing more nutrients to root cultures in a mist reactor is to increase the misting duty cycle, ω (min hr^{-1}) which increases the medium flow rate, Q_L, (mL min^{-1}), and thus, the total delivery of nutrients to the roots. The mist duty cycle, however, can significantly affect the growth rate of roots in a mist bioreactor (Towler et al., 2006; Liu et al., 1999) with some evidence of species specificity (DiIorio et al., 1992b). Recent experiments by Towler et al. (2007) separated the effect of duty cycle from concurrent changes in the sugar deposition rate. By varying the mist duty cycle and the sucrose concentration together so that the average sugar deposition rate was constant, they showed that alteration of the mist duty cycle had no significant effect on *A. annua* root growth. In contrast, when different sugar concentrations were fed using the same misting cycle, then growth increased as sugar concentration increased (Towler et al., 2007). Taken together these studies showed that α and C_S are the two most important factors to optimize in order to achieve high growth rates of hairy roots in mist reactors.

Other Factors Affecting Root Growth in Mist Reactors

Besides α, C_S , and ω from the mist deposition model, other factors have been observed to affect root growth in mist reactors including mist flow orientation, inoculum distribution, gas composition, medium "conditioning" factors, and medium preparation methods. Mist reactors that are fed from the top down have the advantage of co-current down-flow of gas and liquid phases along with gravity and this facilitates drainage. If mist is provided from the bottom up, coalescence is greatest on the roots closest to the mist feed so less mist reaches tissues in the top layers of the growth chamber (Liu et al., 1999). With increasing root bed density, the lower regions will likely become waterlogged if mist is provided upwards. Indeed using upflow mist delivery (Chatterjee et al., 1997) resulted in root necrosis in *A. annua* a condition that was never observed with downflow mist delivery (Wyslouzil et al., 2000). It is also likely that as the root bed becomes very dense, the lower sections will accumulate even more liquid and essentially become submerged.

If the root inoculum is not evenly distributed within the growth chamber of a mist reactor, a large percentage of the mist will exit the chamber without contacting any roots. Furthermore, roots that capture mist droplets will probably grow rapidly and subsequently form dense root clusters. Most mist reactors have been inoculated by first flooding the growth chamber with media, adding the roots, and then running the reactor as a bubble column until the roots immobilize onto a trellis.

Like roots of whole plants, hairy roots also respond to changes in gas composition with their response varying with plant species. Besides oxygen most other gas studies have focused mainly on CO_2. Air enriched with CO_2 enhanced growth by decreasing the lag time in some root cultures (30), but not in others (Wyslouzil et al., 2000; Kim et al., 2002). If CO_2 is observed to stimulate hairy root growth for a species under study, it is important not to overaerate a root bed in a mist reactor to avoid purging beneficial gases. Alternatively, the ability to provide a controlled amount of CO_2 enriched air to root beds is one of the design benefits of using a mist reactor. The effect of changing the combined levels of O_2, CO_2, or C_2H_4 has not, however, been studied to date.

Reactors can be operated in a batch mode where coalesced mist culture medium is collected and recycled back into the reservoir after exiting the growth chamber. They can also be run in a continuous mode whereby the culture medium only passes over the tissue once prior to being discarded, and fresh medium is continuously added to the reservoir. Although the latter supplies fresh medium and would be expected to stimulate growth, usually it does not because roots often prefer "conditioned" medium. Conditioned medium is produced by growing roots in fresh media for up to 2 weeks (Wyslouzil et al., 2000) during which time they adjust the pH and distribution of nutrients. Roots also exude growth stimulating compounds including oligosaccharides (Schroder et al., 1989), peptides (Matsubayashi et al., 1996), and auxins (Weathers et al., 2005). Conditioned medium can easily be introduced into the medium reservoir by filter sterilization.

Most reactor studies use medium prepared by autoclaving. During this process however, the sucrose in the medium is partially hydrolyzed to fructose and glucose, and small amounts of toxic byproducts are produced (Weathers et al., 2004) resulting in an undefined medium. Such variations can lead to aberrations in hairy root growth (Kim et al., 2002) and secondary metabolite production (Kim et al., 2002; Weathers et al., 2004) in both liquid and gas-phase culture systems. Use of filter sterilized medium eliminates such problems.

SCALING UP MIST/SPRAY REACTORS

Despite many lab scale studies, there have been few in depth studies on scaling up mist or spray reactors for hairy root cultures. One early study at 500 L used a series of hanging hooks to immobilize *Datura* roots that were then grown for 40 days (Wilson, 1997). Productivity, however, was low at 1.3 g DW L^{-1} d^{-1} with a final packing density of about 8% (α = 0.08; FW V^{-1}). Three more recent studies had more productive growth yields (Williams and Doran, 2000; Ramakrishnan and Curtis, 2004; Huang and Chou, 2006), and the key elements of those studies are summarized in Table 4. In contrast to the fine mists (droplets~10 µm) used by the Weathers and Liu labs (Table 2), the studies shown in Table 4 used sprays, with median volume droplet diameters exceeding 50 µm. Williams and Doran (2000) concluded that their spray reactor was not effective because as the biomass density of the root bed increased the large droplets resulted in the root bed becoming water logged and gas exchange plummeted. Indeed Ramakrishnan and Curtis et al. (2004) noted that in their spray reactor large amounts of interstitial liquid accumulated, and they needed to enrich the aeration gas with oxygen to maintain growth. Furthermore, the liquid feed rate (L d^{-1}) in these 3 studies often exceeded the volume of the culture vessel further aggravating liquid accumulation. For example, in a 14 L vessel Ramakrishnan and Curtis (2004) provided 648 L d^{-1} of liquid media to a maximum of 752 g FW of roots (Table 4). They initiated their reactors in a liquid bubble column mode for ~12 d, shifted to trickle bed mode for the next 8-9 d to enhance aeration, and eventually enriched the air to 37% O_2 in order to maintain high growth rates during the last 5 days of culture. Only then were they able to achieve a final high yield of root biomass (Ramakrishnan and Curtis, 2004).

Although the 14 L trickle bed reactor run by Ramakrishnan and Curtis (2004) reached a final α = 0.75 (Table 4), the growth rate was 0.13 d^{-1} without oxygen enrichment and 0.21 d^{-1} with oxygen enrichment. In contrast their scaled-down (1.6 L) version of that reactor yielded a higher growth rate of 0.35 - 0.40 d^{-1} for the first 4 days. Unfortunately, throughout the rest of the culture period the growth rate steadily declined to < 0.10 d^{-1}. The small fine mist droplet reactor used by Towler et al. (2007) was much smaller (0.05 L), yet a biomass density comparable to that obtained by Ramakrishnan and Curtis (2004) was attained with an α = 0.71 and overall growth rate of 0.11 d^{-1}.

This was, however, achieved without addition of extra O_2 or other nutrients (Towler et al., 2007). The 1.6 L trickle bed reactor (Ramakrishnan and Curtis, 2004) had a biomass/medium ratio about 8× greater and a bed depth about 5× deeper than the small mist reactor of Towler's, never the less the high packing density obtained in the small mist reactor substantiates the importance of droplet size and total liquid throughput in gas phase reactors. Clearly, however, more equitable performance comparisons must be made to determine the potential of larger scale mist reactors.

In mist reactors, therefore, it is critical to keep droplet diameter small and liquid throughput must not exceed levels that result in water logging of the culture. If the latter occurs, then a mist reactor reverts to a liquid-phase reactor. In the mist reactor high-density root beds grow faster than low density beds, because high-density beds capture media droplets more efficiently (Towler et al., 2007). This counterintuitive reactor, however, poses a challenge to scale-up because the high inoculum levels required to create a dense root bed in a mist reactor quickly become impractical as the reactor volume increases. Taken together it is clear that gas-phase reactors must be carefully designed, studied, and operated to achieve the high growth yields that we (Towler et al., 2007), and others (Suresh et al., 2005; Ramakrishnan and Curtis, 2004), have observed.

CONCLUSIONS AND FUTURE RECOMMENDATIONS

Mist and other gas-phase reactors clearly offer some significant benefits for culturing hairy roots, including lack of oxygen stress, rapid growth, and production of high yields and, sometimes, of new secondary metabolites. Scaling up these reactors however, presents some unique challenges. For example, what is the volumetric limit of a mist reactor? How does root morphology alter performance of a reactor? Do thick hairless roots grow more effectively than thin hairy ones? The mist deposition model (Wyslouzil et al., 1997) suggests that may indeed be the case. If so, how does one engineer a reactor to accommodate variations in root morphology? These types of questions along with others need to be addressed before mist and other gas-phase reactors become practical.

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Table 4. Comparisons between three recently scaled-up gas-phase hairy root reactors.

Reactor parameters/Results	Huang & Chou (2006)	Ramakrishnan & Curtis (2004)	Williams & Doran (2000)
Plant species	*Stizolobium hassjoo*	*Hyoscamus muticus*	*Atropa belladonna*
Working vol (L)	9	14	4.4
Aspect ratio	2.1	6.0	1.7
Median drop diameter (µm) [1]	> 50	> 150	> 50
Medium flow (L min^{-1})	0.12	0.45	0.13
Misting cycle	1 hr on:1 hr off	continuous	continuous
Gas flow (vol gas/working vol/ min (vvm))	0.022	~ 0.7	0.27
Inoculum (g DW L^{-1})	0.15 (est)	0.2	0.18 (est)

Final yields (g): FW L^{-1} α DW L^{-1}	83 (est) 0.083 6.95	752 0.752 36	182 (est) 0.182 11.23 (est)
μ (d^{-1}) [run length in days]	0.20 (est) [16]	0.21 [25]	0.16 (est) [24.9] 0.08 (est) [41.8]
Notes	countercurrent dual misting	cocurrent gas/liquid	cocurrent gas/liquid

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