Plant Cell Reports (2000) 19:407–413 

W.-C. Yu · P.J. Joyce · D.C. Cameron · B.H. McCown

# Sucrose utilization during potato microtuber growth in bioreactors

Received: 1 December 1998 / Revision received: 6 May 1999 · Accepted: 19 May 1999

**Abstract** Potato microtubers are used as pathogen-tested in vitro stocks for certified seed potato production. Microtubers grown in a rotating bioreactor grew at a faster rate when the medium was replaced frequently. Although the total microtuber number was not affected, the number of microtubers over 1 g quadrupled when 75% of the medium was replaced every 2 weeks when compared with no medium refreshment. Significantly slower microtuber growth rates resulted when a lower sugar concentration (40 g $l^{-1}$ instead of 80 g $l^{-1}$) was used or when a mixture of glucose and fructose replaced sucrose. Although high sucrose levels are necessary for optimal microtuber production, the sucrose supplied was rapidly hydrolyzed into glucose and fructose, making the long-term maintenance of desirable sucrose levels difficult. These results indicate that successful strategies to reduce sucrose hydrolysis without inhibiting microtuber growth will improve the efficiency of sucrose utilization in potato microtuber bioreactors.

**Key words** *Solanum tuberosum L.* · In vitro tuberization · Micropropagation · Invertase

**Abbreviations** *HPLC*: High performance liquid chromatography · *MS*: Murashige and Skoog (1962) medium

Communicated by G. Phillips

W.-C. Yu · D.C. Cameron
Department of Chemical Engineering, University of Wisconsin, Madison, WI 53706, USA

B.H. McCown (✉)
Department of Horticulture, University of Wisconsin, Madison, WI 53706, USA
e-mail: bhmccown@facstaff.wisc.edu

P.J. Joyce
Small Potatoes, Inc, 320 Russell Street, Madison, WI 53704, USA

## Introduction

Microtubers, which are potato tubers produced in vitro, have many properties that make them ideal propagules for producing high-quality seed potatoes. Since microtubers are produced in vitro, they usually stay pathogen-free if started with pathogen-free materials. Compared with the other in vitro potato propagule, microshoots, microtubers are more robust, easier to handle, and more amenable to automatic planting (McCown and Joyce 1991). Because microtubers can be stored for extended periods, they also provide more flexible planting options. When more generations of multiplication are performed in vitro, fewer generations are needed in the field. The lower dependence on field multiplication reduces the risk of disease infection and thus can improve the health status of certified seed potatoes. The reduced risk of disease build-up in potato stocks is particularly important in regions with prevailing high disease pressures.

Although microtubers can be used in the greenhouse to produce minitubers (seed tubers produced under greenhouse conditions) or transplants for field planting, direct field seeding using microtubers has high commercial potential, particularly in regions with warm, well-drained soils during the planting season. However, the size of the tubers destined for field plantings has a strong effect on the performance of the resulting potato crop. Studies with direct field planting of microtubers (Haverkort 1991) and minitubers (Lommen and Struik 1994) have shown that small tubers emerge slower and with less uniformity, establish their canopy more slowly, intercept less radiation, and yield less than plants established from conventional seed tubers. In order for the tuber to perform adequately through the rigorous conditions required for commercial seed potato production, a minimum fresh weight of 0.5 g is necessary (Lommen and Struik 1994; Struik and Lommen 1990). Larger sized seed tubers perform better with less risk, but 0.5 g is a minimum fresh weight for commercially viable yields.

The minimum fresh weight requirement (0.5 g) excludes most published microtuber production methods from becoming directly commercially viable for direct field planting. Most systems have produced microtubers with a median fresh weight below 0.5 g (Garner and Blake 1989; Hussey and Stacey 1984; Rosell et al. 1987; Seabrook et al. 1993; Tovar et al. 1985; Wang and Hu 1982; Wattimena 1983). A few reported systems produce microtubers with an average fresh weight greater than 0.5 g, but less than 1.0 g (Akita and Takayama 1994a; Leclerc et al. 1994; Oka and Sluis 1996).

The environment and medium within the in vitro growth vessel influences the size of the microtubers. Such environmental factors include photoperiod, temperature, gaseous components such as ethylene and $CO_2$, and medium components (Mingo-Castel et al. 1976; Wang and Hu 1985). One of the most important factors is the carbon source in the medium; both its type and concentration have profound effects on tuber growth. Sucrose is considered to be the optimal carbon source as compared to its constituent hexoses–glucose and fructose (Dodds et al. 1992; Khuri and Moorby 1995). Using radiolabeled sugars, Khuri and Moorby (1995) demonstrated that more sugar is translocated to microtubers when sucrose, rather than glucose or fructose, is the carbon source. The reported optimal concentration of sucrose ranges from 60 to 80 g $l^{-1}$ (Abbot and Belcher 1986; Dodds et al. 1992; Garner and Blake 1989; Hussey and Stacey 1984; Lawrence and Baker 1963). A too high or too low concentration of sucrose leads to slower tuberization and fewer and smaller microtubers.

Sucrose may play a dual role in microtuber development. Apart from being a suitable carbon source that is easily assimilated by the microplants and converted to starch in developing microtubers, sucrose, at a concentration of 80 g $l^{-1}$, also provides a favorable osmolarity for microtuber development (Khuri and Moorby 1995).

Since microtuber growth is strongly affected by sugar type and concentration, an understanding of how these two factors change over time is important for the optimal operation of microtuber production systems. Although sucrose is susceptible to partial or total hydrolysis in potato microtuber bioreactors (Dodds et al. 1992; Akita and Takayama 1994a), little is known concerning the rate of sucrose utilization and hydrolysis in such systems nor the effects on the efficiency of microtuber production. In order to determine these relationships, we studied in vitro tuberization and tuber growth in a rotating bioreactor. We first examined the effect of medium refreshment on microtuber growth. Then, to determine if rapid sucrose hydrolysis would limit microtuber growth even with medium refreshment, we performed another set of experiments with various types and concentrations of sugar.

## Materials and methods

### In vitro shoot multiplication

In vitro potato shoots *(Solanum tuberosum* L. 'Russet Burbank') were routinely subcultured every 4–5 weeks by placing 8 single node cuttings in each culture vessel. The culture was maintained at 22°±2 °C under 80 μE $m^{-2}$ $s^{-1}$ of continuous fluorescent lighting in 280-ml glass jars covered with polypropylene "B" caps (Magenta Corporation, Chicago, Ill.). Each jar contained 30 ml of solid MS medium (pH 5.6) (Murashige and Skoog 1962) supplemented with 20 g $l^{-1}$ sucrose, 1.3 g $l^{-1}$ calcium gluconate, 3.0 g $l^{-1}$ agar, and 1.1 g $l^{-1}$ Gelrite (Kelco, Rahway, N.J.).

### In vitro tuberization

The bioreactors were made of 1.0-l polycarbonate centrifuge bottles (Nalgene, Rochester, N.J.). Two ports were provided for air flow: one in the cap and one at the bottom of the bottle. A 0.2-μm Teflon filter was fitted on each port to maintain sterility. A specially constructed apparatus (Fig. 1) was used to both rotate the bottles and simultaneously provide a forced air flow through the bottles. The apparatus consisted of a driving unit with a gear motor and a chain, a hollow shaft with air regulators and, fixed to the shaft, two 60-cm diameter wooden boards each cut with 20 circular holes whose diameter of 10 cm closely fit the outside diameter of the bottles. One end of the shaft was sealed, and the other was fitted with a swivel fitting which was connected to a compressed air line. Thus, the hollow shaft also acted as a small pressurized chamber, through which air was delivered from an oil-free air compressor to the bottles.

A sequential two-stage growth regime was used throughout the studies. First, the elongation stage, for shoot and root growth, was conducted under constant lighting at 22 °C with no rotation and 50 ml $min^{-1}$ of air flow. Each bioreactor contained 50 single nodal cuttings and 100 ml of liquid MS medium supplemented with 30 g $l^{-1}$ of sucrose. After 3 weeks of growth in this environment, the resulting potato shoots (now about 10–15 cm long) were then subjected to tuberization conditions. The residual shoot growth medium was drained, and 200 ml of tuberization medium was added. Tuberization and subsequent tuber growth was conducted in the dark at 18 °C with 0.5 rpm rotation and 150 ml $min^{-1}$ of air flow. The standard liquid tuberization medium contained MS salts and vitamins, 170 μM coumarin, and 80 g $l^{-1}$ sucrose. Other sugars and concentrations were used for specific experiments.

### Effect of medium refreshment

Three different medium supply levels were investigated, equivalent to 0%, 10%, and 75% of medium refreshment every 2 weeks. The time courses of tuberization, tuber growth, and sugar concentration were followed by randomly harvesting five replicate bioreactors from each treatment every 2 weeks over a period

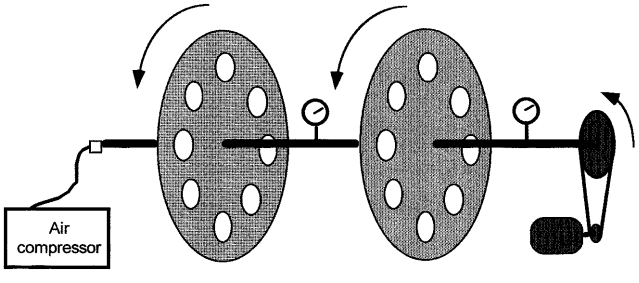


**Fig. 1** Rotating apparatus for 1-l rotating bioreactors

of 10 weeks. The number and fresh weight of microtubers were recorded. Sugar concentrations were measured by a Waters HPLC system (Milford, Mass.) equipped with a Sugar-Pak column (Waters, Milford, Mass.) and a refractometer. The column was maintained at 90 °C with the mobile phase of an aqueous solution of 0.5 g $l^{-1}$ Ca-EDTA flowing at 0.5 ml $min^{-1}$.

### Effect of sugar type and concentration

Two weeks after tuber initiation with the standard tuberization medium, bioreactors were subjected to four different medium treatments consisting of two types of sugar at two total concentrations. These treatments were 40 and 80 g $l^{-1}$ of sucrose, and 40 and 80 g $l^{-1}$ of a 1:1 (mol basis) mixture of glucose and fructose. The treatment media contained only half the normal amount of MS salts and vitamins. In an effort to maintain near-constant nutrient levels, the medium in all bioreactors was drained and replaced every 2 days. Bioreactors were harvested after 6 weeks of microtuber growth, or 4 weeks after the treatment began. Plants were separated into microtubers, shoots, and roots, and were weighed. Sugar concentrations in the media were determined by HPLC as described above.

### Experimental design

Treatment bioreactors were randomized as to position on the rotating apparatus. Each bioreactor was considered a replicate, and each treatment bioreactor was replicated 5 times in each experiment. All data was included except when a bioreactor was contaminated. Because of the extensive time and labor required to conduct each experiment, experiments were not repeated in time. Descriptive statistics were performed using SigmaStat 2.0 (Jandell Corp, San Rafael, Calif.).

## Results and discussion

### Effect of medium refreshment

The total fresh weight of microtubers was markedly enhanced by medium refreshment. Microtubers grew very little after 6 weeks in the no-refreshment treatment, whereas at the 75% medium refreshment level, the growth of microtubers was linear with no sign of decline in growth rate (Fig. 2). If allowed, the growth would most likely have continued beyond 10 weeks.

The enhanced microtuber growth from medium refreshment was not the result of an increase in the number of microtubers, but instead was due to an increase in the number of larger microtubers (Fig. 3). As shown in Fig. 3A, the total number of microtubers $\geq 0.01$ g in fresh weight reached about 100 in 2 weeks, and did not change significantly afterwards. Thus, tuber initiation was completed within 2 weeks, and no significant number of new tubers was formed in response to any of the medium refreshment treatments. The number of larger sized microtubers ($\geq 0.5$ g), on the other hand, increased with increasing medium refreshment, especially microtubers in the size class larger than 1.0 g (Fig. 3B). With 75% medium refreshment, $40.4 \pm 0.2$ ($n = 5$) microtubers over 1.0 g were produced from 50 single nodal cuttings in 10 weeks; over 80% of these explants produced microtubers larger than 1.0 g. This was nearly a fourfold improvement over the case with no medium refreshment ($11.2 \pm 1.1$ microtubers over 1.0 g). Further increases in the number of microtubers over 1.0 g was likely if the experiment had been allowed to continue beyond week 10.

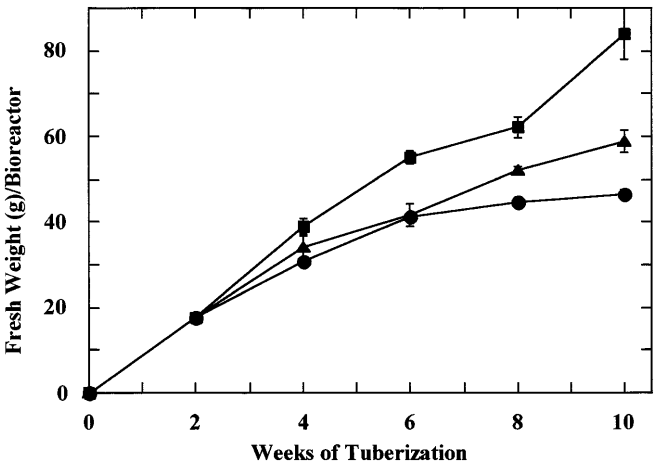


**Fig. 2** Effect of medium refreshment on potato microtuber growth. Each bioreactor was inoculated with 50 single nodal cuttings and 100 ml of shoot growth medium. Three weeks later, the resulting shoots were tuberized with 200 ml of 8% sucrose tuberization medium. Medium refreshment was performed every 2 weeks at three different levels – 0% (●), 10% (▲), and 75% (■). Data points represent means of four to five replicate bioreactors, and error bars are ±SE

Two distinct phases can be distinguished in the rotating bioreactor system used here: (1) microtuber initiation and (2) the further growth and development of the initiated microtubers. These distinct phases were also observed in the jar fermentor system of Akita and Takayama (1994b). The medium refreshment treatments may not have influenced total microtuber number because these treatments were not begun until after microtuber initiation had already occurred.

With no medium refreshment, the total sugar concentration steadily decreased as microtuber growth proceeded (Fig. 4). By week 6, when the growth of microtubers had nearly stopped, less than 20 g $l^{-1}$ of total sugar was left. The sucrose concentration, on the other hand, showed a sharp drop from 80 to 6 g $l^{-1}$ within the first 2 weeks of microtuber growth, even though there was still 50 g $l^{-1}$ of total sugar left (Fig. 4). Most of the sucrose had been converted into glucose and fructose (Fig. 4). Even with 75% medium refreshment every 2 weeks, the sucrose concentration was zero at the end of each 2-week period (Fig. 5A), although the total sugar concentration was maintained between 40 and 80 g $l^{-1}$ (Fig. 5B).

The hydrolysis of sucrose leads to the production of equal amounts of glucose and fructose. However, the glucose concentration was always lower than the fructose concentration (Fig. 4), suggesting that the potato plant has a slightly greater preference for the absorption of glucose than fructose.

Proper medium supply is essential for continued microtuber growth and appears to be a key for

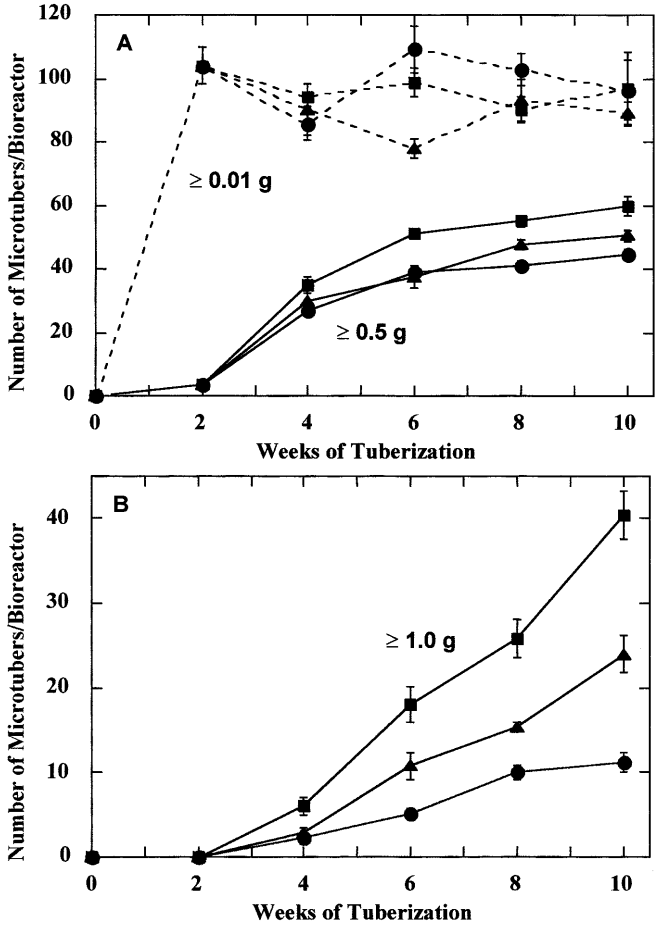


**Fig. 3A, B** Number of potato microtubers in various size categories during tuberization with medium refreshment every 2 weeks at 0% (●), 10% (▲), and 75% (■). **A** Fresh weights ≥0.01 g (*dashed line*) and ≥0.5 g (*solid line*), **B** fresh weights ≥1.0 g. Each bioreactor was inoculated with 50 single nodal cuttings and 200 ml of tuberization medium. Data points represent means of four to five replicate bioreactors, and error bars are ±SE

achieving large microtubers. High total sugar concentrations and a uniform availability of sucrose probably both play important roles, based on previous reports that sucrose is the most effective carbon source for in vitro tuber growth (Dodds et al. 1992; Khuri and Moorby 1995), and the optimal level is about 60–80 g $l^{-1}$ (Abbot and Belcher 1986; Dodds et al. 1992; Garner and Blake 1989; Hussey and Stacey 1984). Unfortunately, the sucrose supplied in the medium for potato microtuber growth is rapidly hydrolyzed into glucose and fructose; such sucrose degradation has the potential to severely affect subsequent microtuber growth and limit the effectiveness of medium replenishment in bioreactors.

## Effect of sugar type and concentration

To fully investigate whether the rapid hydrolysis of sucrose during microtuber growth would lead to

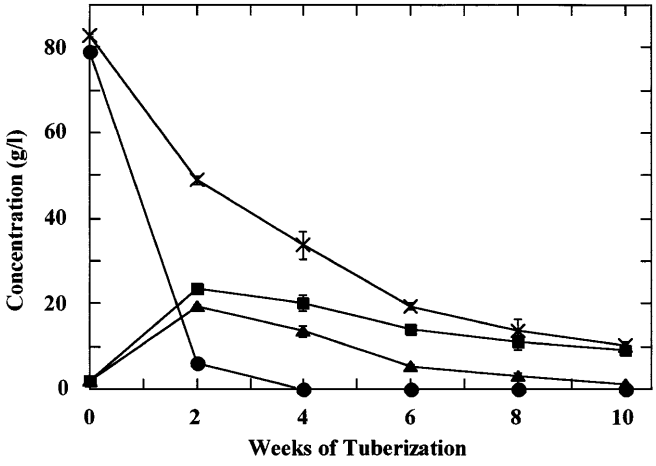


**Fig. 4** Concentrations of sucrose (●), glucose (▲), fructose (■), and total sugar (×) during 10 weeks of potato tuberization with no medium refreshment. Each bioreactor was inoculated with 50 single nodal cuttings and 200 ml of tuberization medium. Data points represent means of four to five replicate bioreactors, and error bars are ±SE

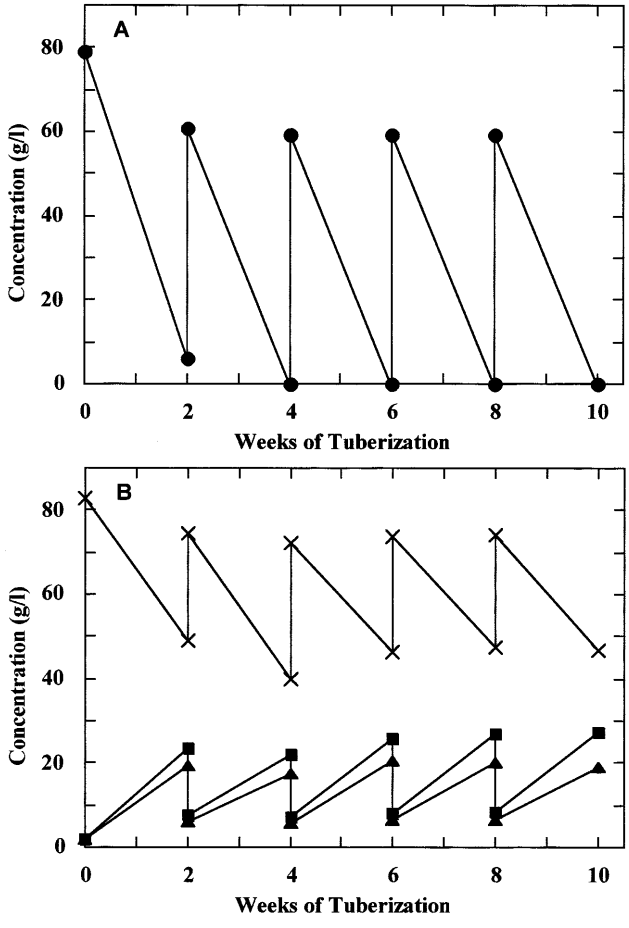


**Fig. 5A, B** Concentrations of sucrose (●) (**A**) and glucose (▲), fructose (■), and total sugar (×) (**B**) during 10 weeks of potato tuberization with 75% medium refreshment every 2 weeks. Each bioreactor was inoculated with 50 single nodal cuttings and 200 ml of tuberization medium. Data points represent means of four to five replicate bioreactors

reduced microtuber size, a comparison of microtuber growth on sucrose and its hydrolyzed product, a 1:1 (mol basis) mixture of glucose and fructose, is needed. To our knowledge, such a study has not been conducted previously. In addition, the effect of carbon source on the initiation of microtubers and on the subsequent microtuber growth has often been confounded in previous research. Several reports have investigated the effect of carbon source on microtuber culture (Abbot and Belcher 1986; Dodds et al. 1992; Garner and Blake, 1989; Hussey and Stacey 1984; Khuri and Moorby 1995; Lawrence and Barker 1963) and have shown that sucrose at about 80 g $l^{-1}$ is more stimulatory than other sugars and concentrations. However, in these reports plant materials were subjected to various levels and types of sugar from the beginning of tuberization, and thus the results reflect the combined effects on both microtuber initiation and subsequent growth and development of induced microtubers.

To determine how much the rapid hydrolysis of sucrose adversely affects the growth of initiated microtubers, we compared microtuber growth under four different conditions: 40 and 80 g $l^{-1}$ of sucrose and 40 and 80 g $l^{-1}$ of 1:1 (mol basis) glucose/fructose mixture. In terms of the total microtuber weight achieved and the number of larger sized ($\geq$0.5 g and $\leq$1.0 g) microtubers produced, the 80 g $l^{-1}$ sucrose treatment clearly outperformed the other three treatments (Table 1). Lowering the sucrose concentration to 40 g $l^{-1}$ not only resulted in less growth in microtubers but also shifted the growth towards shoots and roots, as indicated by the lower microtuber biomass/total biomass ratio. Such a shift in biomass partitioning was also observed when the concentration of glucose and fructose was decreased. However, unlike the case with sucrose where the number of larger sized microtubers increased with increasing sugar concentration, the 80 g $l^{-1}$ glucose/fructose treatment actually produced fewer microtubers over 1.0 g than the 40 g $l^{-1}$ glucose/fructose treatment. Such a tendency to produce small microtubers may be the result of the unfavorably high osmolarity (444 mM) generated by 80 g $l^{-1}$ of glucose/fructose as compared to the 80 g $l^{-1}$ of sucrose (234 mM osmolarity). These results clearly demonstrate that sucrose is a superior carbon source for microtuber growth than its hydrolyzed products, glucose and fructose, and that sucrose availability is a major factor in determining microtuber size.

To achieve optimal microtuber sizing, sucrose concentrations should be maintained at the optimal level throughout the microtuber development period. However, maintaining 80 g $l^{-1}$ sucrose in the microtuber bioreactor environment is difficult due to rapid hydrolysis of sucrose. At week 3, about 30 g $l^{-1}$ of sucrose had been hydrolyzed in 2 days for both the 40 g $l^{-1}$ and 80 g $l^{-1}$ sucrose treatments, while little change was observed in the total sugar concentration (Fig. 6). As microtuber growth proceeded, even more sucrose was degraded during the 2 days between medium replacement (Fig. 6). In the 40 g $l^{-1}$ treatment, at week 4 the sucrose concentration dropped to zero in 2 days; in the 80 g $l^{-1}$ sucrose treatment, at week 6 only 15 g $l^{-1}$ of sucrose was left 2 days after refreshment. In all treatments, the decrease in the total sugar concentration remained relatively small; for example, less than 10 g $l^{-1}$ of sugar was consumed in 2 days in the 80 g $l^{-1}$ sucrose treatment at week 6. These observations demonstrate that sucrose was degraded at a much faster rate than the uptake rate of sugars into the cultured potato tissues. In the 80 g $l^{-1}$ case, the rate of sucrose hydrolysis was estimated to be about 6 times the uptake rate of sugars. Such a rapid hydrolysis of sucrose makes its utilization as the carbon source in potato bioreactors very inefficient.

## Sucrose hydrolysis and sucrose-hydrolyzing enzymes

Sucrose is frequently used as a carbon source for plant tissue culture. Its hydrolysis into glucose and fructose has been reported in a wide variety of plant cell and tissue cultures (George 1993). Examples include suspension cell cultures of sugar cane (Thom et al.

**Table 1** Effect of sugar type and concentration on potato microtuber growth. Each bioreactor was inoculated with 50 single nodal cuttings and 200 ml of 8% sucrose tuberization medium. After 2 weeks of tuberization in the 8% sucrose medium, 100% medium replacement was performed every 2 days using media containing various types and concentrations of sugars as indicated below. Data are presented as mean ± SE ($n$=4 or 5) (*FW* fresh weight)

| Characteristics per bioreactor | Treatment | | | |
|---|---|---|---|---|
| | 40 g/l sucrose | 80 g/l sucrose | 40 g/l G+F | 80 g/l G+F |
| Total microtuber FW (g) | 46.5 ± 2.7 | 60.8 ± 3.6 | 36.8 ± 2.0 | 41.1 ± 3.0 |
| Increase in total microtuber FW[a] (g) | 28.7 ± 3.8 | 43.0 ± 4.6 | 19.0 ± 3.0 | 23.3 ± 4.0 |
| Ratio of increase in FW, microtuber/total biomass[b] | 0.21 ± 0.02 | 0.46 ± 0.03 | 0.31 ± 0.02 | 0.57 ± 0.03 |
| Number of microtubers ≥ 0.01 g | 81.3 ± 1.1 | 102.0 ± 1.6 | 87.8 ± 0.9 | 133.4 ± 6.6 |
| Number of microtubers ≥ 0.5 g | 41.5 ± 3.2 | 51.5 ± 3.2 | 31.8 ± 2.5 | 29.8 ± 2.4 |
| Number of microtubers ≥ 1.0 g | 13.8 ± 1.4 | 21.4 ± 1.2 | 9.0 ± 0.5 | 4.4 ± 0.4 |

[a] The increase in total microtuber fresh weight during treatment, i.e., between 2 and 6 weeks of tuberization

[b] Total biomass includes shoot, root, and microtuber

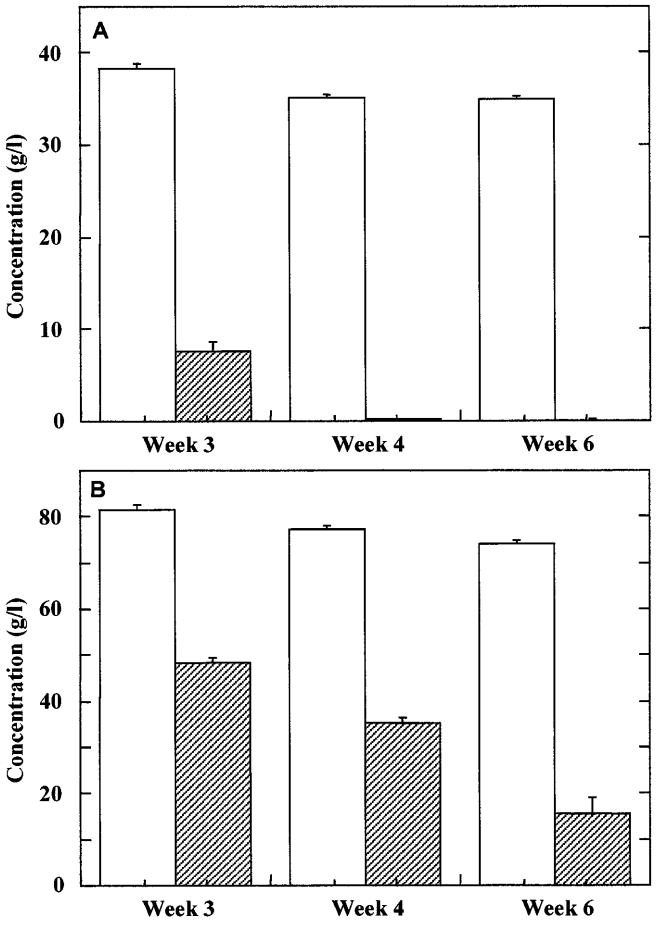


**Fig. 6** Total sugar (*shaded bar*) and sucrose (*clear bar*) concentrations 2 days after 100% medium replacement during 3–6 weeks of potato tuberization. Each bioreactor was inoculated with 50 single nodal cuttings and 200 ml of 8% sucrose tuberization medium. After 2 weeks of tuberization under the 8% sucrose medium, 100% medium replacement was performed every 2 days using media containing either 40 g $l^{-1}$ (**A**) or 80 g $l^{-1}$ (**B**) of sucrose. Data points represent means of four to five replicate bioreactors and error bars are ± SE

1981), carrot (Kanabus et al. 1986), sugar beet (Masuda et al. 1988) and asparagus (Daie et al. 1987); root cultures of tomato (Weston and Street 1968) and strawberry (Nuutila et al. 1997); shoot and organ cultures of rose (Riek et al. 1997), carnation (Woo and Park 1993), and orchids (Hew et al. 1988). Akita and Takayama (1994a) also reported that in their potato microtuber jar fermentor, all the remaining sugars were glucose and fructose after 10 weeks of in vitro growth.

Two enzymes can catalyze the hydrolysis of sucrose in plants: sucrose synthase and invertase (Avigad 1982; Hawker 1985; Copeland 1990). Two forms of invertase exist, and these vary in their pH optimums. One form, termed acid invertase, has an optimal activity near pH 5, while the other form, termed neutral or alkaline invertase, has a maximum activity at about pH 7 (Avigad 1982; Hawker 1985; Copeland 1990). All of these enzymes are present in the potato plant (Mares et al. 1985; Rees and Morrell 1990). While acid invertase can be located in vacuoles, the cell wall, and the free space outside cells, both alkaline invertase and sucrose synthase are generally believed to be cytoplasmic enzymes (Avigad 1982; Hawker 1985; Copeland 1990). For intracellular enzymes to be involved in the hydrolysis of sucrose in a culture medium, an unlikely cycle of sucrose uptake and subsequent secretion of glucose and fructose is necessary. Extracellular acid invertase, on the other hand, is capable of sustaining direct sucrose hydrolysis in the medium. In varied systems, cell-wall-located acid invertase is responsible for sucrose hydrolysis in the medium (Daie et al. 1987; Kanabus et al. 1986; Masuda et al. 1988; Thom et al. 1981; Weston and Street 1968), while the involvement of intracellular enzymes has not been demonstrated.

Sucrose is taken-up unaltered by tomato roots (Chin and Weston 1975), sugar beet root tissues (Giaquinta 1977; Wyse 1979; Lemoine et al. 1988), and asparagus cells (Daie et al. 1987), despite the presence of active cell-wall acid invertase in these systems. Tomato roots exhibit better growth on sucrose than glucose and fructose (Weston and Street 1968), and this has been attributed to their inability to take up hexoses efficiently (Chin et al. 1981). Nevertheless, the acid invertase in the cell wall of tomato roots causes the conversion of sucrose to hexoses in the medium. The physiological role of extra-cellular acid invertase remains unclear.

In our microtuber system, preliminary tests showed no detectable sucrose hydrolyzing activity in the medium (data not shown), indicating that the enzyme is associated with plant materials. It is probable that a cell wall associated acid invertase is responsible for the rapid degradation of sucrose observed in potato cultures.

In conclusion, we found that a key to producing large microtubers in bioreactors is maintaining an adequate supply of sucrose throughout the microtuber growth stage. A relatively high concentration of 80 g $l^{-1}$ must be maintained for optimal microtuber growth. However, maintaining a high sucrose concentration is very difficult because of the very rapid hydrolysis of sucrose in potato bioreactors. Methods to inhibit sucrose hydrolysis while maintaining microtuber growth would greatly improve the efficiency of carbon assimilation in bioreactor systems.

**Acknowledgments** This study was funded in part by the United States Department of Agriculture Small Business Innovation Research grant program, by the UW System Applied Research Grant Program, and by the UW-Madison Agriculture Experimental Station and the College of Engineering, University of Wisconsin-Madison.

## References

Abbot AJ, Belcher AR (1986) Potato tuber formation in vitro. In: Withers LA, Alderson PG (eds) Plant tissue culture and its agricultural applications. Butterworths, London, pp 113–122

Akita M, Takayama S (1994a) Stimulation of potato *(Solanum tuberosum* L.) tuberization by semicontinuous liquid medium surface level control. Plant Cell Rep 13:184–187

Akita M, Takayama S (1994b) Induction and development of potato tubers in a jar fermentor. Plant Cell Tissue Organ Cult 36:177–182

Avigad G (1982) Sucrose and other dissaccharides. In: Loewus FA, Tanner W (eds) Encyclopedia of plant physiology, vol. 13:plant carbohydrates I, part A: intracellular carbohydrates. Springer, Berlin Heidelberg New York, pp 217–347

Chin CK, Weston GD (1975) Sucrose absorption and synthesis by excised *Lycopersicon esculentum* roots. Phytochemistry 14:69–70

Chin CK, Haas JC, Still CC (1981) Growth and sugar uptake of excised root and callus of tomato. Plant Sci Lett 21:229–234

Copeland L (1990) Enzymes of sucrose metabolism. In: Lea PJ (ed) Methods in Plant Biochemistry, vol. 3:enzymes of primary metabolism. Academic Press, London San Diego, pp 73–85

Daie A, Chin CK, Pitcher L (1987) Differential rates of sucrose and hexose transport by asparagus cell cultures. Plant Sci 53:101–107

Dodds JH, Silva-Rodriguez D, Tovar P (1992) Micropropagation of potato *(Solanum tuberosum* L.) In: Baja YSP (ed) Biotechnology in agriculture and forestry, vol. 19:high-tech and micropropagation III. Springer, Berlin Heidelberg New York, pp 91–106

Garner N, Blake J (1989) The induction and development of potato microtubers in vitro on media free of growth regulating substances. Ann Bot 63:663–674

George EF (1993) Plant propagation by tissue culture, part 1. The technology. Exegetics Ltd, England

Giaquinta R (1977) Sucrose hydrolysis in relation to phloem translocation in *Beta vulgaris.* Plant Physiol 60:339–343

Haverkort AJ, Waart M van de, Marinus J (1991) Field performance of potato microtubers as propagation material. Potato Res 34:353–364

Hawker JS (1985) Sucrose. In: Dey PM, Dixon RA (eds) Biochemistry of storage carbohydrates in green plants. Academic Press, London, pp 1–51

Hew CS, Ting SK, Chia TF (1988) Substrate utilization by *Dendrobium* tissues. Bot Gaz 149:153–157

Hussey G, Stacey NJ (1984) Factors affecting the formation of in vitro tubers of potato *(Solanum tuberosum* L.) Ann Bot 53:565–578

Kanabus J, Bressan RA, Carpita NC (1986) Carbon assimilation in carrot cells in liquid culture. Plant Physiol 82:363–368

Khuri S, Moorby J (1995) Investigation into the role of sucrose in potato cv. Estima microtuber production in vitro. Ann Bot 75:295–303

Lawrence CH, Baker WG (1963) A study of tuberization in the potato, *Solanum tuberosum*. Am Potato J 40:349–356

Leclerc Y, Donnelly DJ, Seabrook JEA (1994) Microtuberization of layered shoots and nodal cuttings of potato: the influence of growth regulators and incubation periods. Plant Cell Tissue Organ Cult 37:113–120

Lemoine R, Daie J, Wyse R (1988) Evidence for the presence of a sucrose carrier in immature sugar beet tap roots. Plant Physiol 86:575–580

Lommen WJM, Struik PC (1994) Field performance of potato minitubers with different fresh weights and conventional seed tubers: crop establishment and yield formation. Potato Res 37:301–313

Mares DJ, Sowokinos JR, Hawker JS (1985) Carbohydrate metabolism in developing potato tubers. In: Li PH (ed) Potato physiology. Academic Press, New York, pp 279–327

Masuda H, Toshimasa T, Sugawara S (1988) Acid and alkaline invertases in suspension cultures of sugar beet cells. Plant Physiol 86:312–317

McCown BH, Joyce PJ (1991) Automated propagation of microtubers of potato. In: Vasil IK (ed) Scale-up and automation in plant propagation. Academic Press, New York, pp 95–109

Mingo-Castel AM, Smith OE, Kumamoto J (1976) Studies of the carbon dioxide promotion and ethylene inhibition of tuberization in potato explants cultured in vitro. Plant Physiol 57:480–485

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15:473–497

Nuutila AM, Lindqvist A-S, Kauppinen V (1997) Growth of hairy root cultures of strawberry *(Fragaria* x *ananassa* Duch.) in three different types of bioreactors. Biotechnol Techn 11:363–366

Oka I, Sluis C (1996) Method for producing potato microtubers. US Patent no 5,498,541

Rees T, Morrell S (1990) Carbohydrate metabolism in developing potatoes. Am Potato J 67:835–847

Riek J de, Piqueras A, Debergh PC (1997) Sucrose uptake and metabolism in a double layer system for micropropagation of *Rosa multiflora*. Plant Cell Tissue Organ Cult 47:269–278

Rosell G, de Bertoldi FG, Tizio R (1987) In vitro mass tuberisation as a contribution to potato micropropagation. Potato Res 30:111–116

Seabrook JEA, Coleman S, Levy D (1993) Effect of photoperiod on in vitro tuberization of potato *(Solanum tuberosum* L.) Plant Cell Tissue Organ Cult 34:43–51

Struik PC, Lommen WJM (1990) Production, storage and use of micro- and minitubers. In: MacKerron DKL, Edmond HD, Hall D, Kirkman MA, Lang RW, Mackay GR, McRae DC, Oxley SJP, Proc 11th Triennial Conf Eur Assoc Potato Res. EAPR, Edinburgh, UK, pp 122–133

Thom M, Maretzki A, Komor E, Sakai WS (1981) Nutrient uptake and accumulation by sugarcane cell cultures in relation to the growth cycle. Plant Cell Tissue Organ Cult 1:3–14

Tovar P, Estrada R, Schilde-Rentschler L, Dodds JH (1985) Induction and use of in vitro potato tubers. CIP Circular 13:1–5

Wang P, Hu C (1982) In vitro mass tuberization and virus-free seed-potato production in Taiwan. Am Potato J 59:33–37

Wang P, Hu C (1985) Potato tissue culture and its application in agriculture. In: Li PH (ed) Potato physiology. Academic Press, New York, pp 503–577

Wattimena GA (1983) Micropropagation as an alternative technology for potato production. PhD thesis, University of Wisconsin, Madison, Wis., USA

Weston GD, Street HE (1968) Sugar absorption and sucrose inversion by excised tomato roots. Ann Bot 32:521–529

Woo SH, Park JM (1993) Multiple shoot culture of *Dianthus caryophyllus* using mist culture system. Biotechnol Techn 7:697–702

Wyse R (1979) Sucrose uptake by sugar beet tap root tissue. Plant Physiol 64:837–841