3.0 BIOREACTORS FOR PLANT CELL TISSUE AND ORGAN CULTURES (by Shinsaku Takayama)

3.1 Background of the Technique—Historical Overview

Haberlandt\(^1\) first reported plant cell, tissue, and organ cultures in 1902. He separated plant tissues and attempted to grow them in a simple nutrient medium. He was able to maintain these cells in a culture medium for 20 to 27 days. Although these cells increased eleven-fold in the best case, no cell division was observed. Gautheret\(^2\) was the first to succeed in multiplying the cells from the culture in 1934. He used the cambial tissues of *Acer pseudoplatanus, Salix capreae, Sambucus nigra*. After 15 to 18 months in subculture, cell activity ceased. He reasoned that this inactiveness was due to the lack of essential substances for cell division. He suspected that auxin may have been one of the deficient substances. This compound was first reported in 1928 and was isolated by Kogel in the 1930’s. Addition of auxin to the medium prompted plant cell growth. This finding was reported almost simultaneously by Gautheret\(^3\) and White\(^4\) in 1939. Plant cell tissue and organ culture techniques rapidly developed, and in the mid-1950’s another important phytohormone, cytokinins, had been discovered (Miller, Skoog, Okumura, Von Saltza and Strong 1955).\(^15\) By 1962 Murashige and Skoog\(^6\) had reported a completely defined medium which allowed the culture of most plant cells. Their medium has now become the mostly widely used medium in laboratories around the world.

After these initial discoveries and some significant improvements in media, scientific research on the cultivation of plant cell, tissue, and organs shifted to the area of basic physiological research. Industrial applications were also sought in the production of secondary metabolites, clonal plants, and the improvement of various plant tissues.

Plant cell, tissue, and organ culture can be performed by either solid or liquid culture methods, however, in order to scale up the culture to the level of industrial processes, the liquid culture method must be employed.

Recently, pilot bioreactors as large as 20 kl have been constructed in the research laboratories of Japan Tobacco and Salt Co. and in those of Nitto Denko Co. Solid culture methods were used in large scale pilot experiments for the production of tobacco cells, and liquid culture methods were used in the production of Panax ginseng cells. An outstanding example of cell suspension culture in a pilot scale bioreactor (750 l) was the production of shikonins by Mitui Petrochemical Industries. In all these examples, various technologies have been used to improve the productivity of the metabolites.
The technologies include: (i) selection of a high yielding cell strain, (ii) screening of the optimum culture condition for metabolite production, (iii) addition of precursor metabolites, (iv) immobilized cell culture, and (v) differentiated tissue and/or organ culture. The productivity of various metabolites such as ginsenoside, anthraquinones, rosmalinic acid, shikonins, ubiquinones, glutathione, triptolide, etc., reached or exceeded the amount produced by intact plants. To date, the production costs remain very high which is why most of the metabolites are still not produced on an industrial or pilot plant scale. Development of large scale industrial culture systems and techniques for plant cell, tissue, and organs, and the selection of the target metabolites are the chief prerequisites for the establishment of the industrial production of plant metabolites.

Figure 17. The area of plant cell, tissue and organ cultures.
3.2 Media Formulations

The formulation of the medium for plant cell, tissue, and organ culture depend primarily on nutritional requirements. Intact plants grow photoautotrophically in the soil, (i.e., they use CO₂ as the principal carbon source and synthesize sugars by photosynthesis). In the case of aseptic cultures however, establishment of an autotrophic culture is not achieved so that heterotrophic or mixotrophic growth becomes the distinguishing characteristic. Therefore, such cultures require the addition of carbon as an energy source. Given this fact, the culture medium must be formulated as a chemically defined mixture of mineral salts (macro- and microelements) in combination with a carbon source (usually sucrose). In addition to these constituents, organic constituents such as vitamins, amino acids, sugar alcohols, and plant growth regulators are usually added to the medium. Media commonly used are listed in Table 11.

Table 11. Formulations of most frequently used plant tissue culture media

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<tr>
<th>Ingredients (mg l⁻¹)</th>
<th>MS</th>
<th>B5</th>
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<tr>
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<th>Ingredients (mg l⁻¹)</th>
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<th>White</th>
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<td>Ca D-pantothenic acid</td>
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<td></td>
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<tr>
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<td>20,000</td>
<td>20,000</td>
<td>20,000</td>
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<td>IAA</td>
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3.3 General Applications

The most important fields of research for industrial applications, plant cell tissue and organ cultures are clonal propagation and secondary metabolite production. Plants cultivated in vitro have great changes in their morphological features, from cell tissue to differentiated embryo, roots, shoots or plantlets.

Applications to Secondary Metabolite Production. Plant tissue culture is a potential method for producing secondary metabolites. Both shikonins (Fujita and Tabata 1987)\textsuperscript{[7]} and ginseng saponins (Ushiyama et al., 1986)\textsuperscript{[8]} have now been produced on a large scale by this method. However, the important secondary metabolites are usually produced by callus or cell suspension culture techniques. The amounts of some metabolites in the cell have exceeded the amounts of metabolites in the cells of the original plants grown in the soil. So it is expected that cell culturing may be applicable to industrial processes for the production of useful secondary metabolites. It is common knowledge that when a cell culture is initiated and then transferred, the productivity of the metabolite decreases (Kurz and Constabel, 1979).\textsuperscript{[9]} Once productivity decreases, it becomes very difficult to arrest or reverse the decrease. In order to avoid this phenomenon, many cell strains were screened to select those which would maintain metabolite productivity. Some metabolites such as anthocyanins, shikonins, vinca alkaloids, and ubiquinones have been reported to have increased their productivity significantly. Deus-Neumann and Zenk (1984)\textsuperscript{[10]} have checked the stability of the productivity of the selected cell strains reported in the literature and noted that the production of some metabolites such as anthraquinone (Morinda citrifolia), rosmalinic acid (Colius blumei), visnagin (Ammi visnaga), diosgenin (Dioscorea deltoidea), etc., were stable after several subcultures, but some metabolites such as nicotine (Nicotiana rustica), shikonin (Lithospermum erythrorhizon), ajmalicine (Catharanthus roseus), rotenoids (Derris eliptica), anthocyan (Daucus carota), etc., were shown to be unstable after several subcultures.

Clonal Plant Propagation. Plants are propagated clonally from vegetative tissue or organs via bypass sex. Conventional clonal propagation can be performed by leaf or stem cutting and layering or dividing of the plants, however the efficiency is very low. Recently, many plants were propagated efficiently through tissue culture. This technique was first reported in 1960 by G. Morel\textsuperscript{[11]} for the propagation of orchids and since then, many plants have been propagated by tissue culture. Today there are many commercial
tissue culture nurseries throughout the world. Most of these tissue culture nurseries are using flasks or bottles containing agar medium for commercial propagation, but the efficiency is also low. In order to improve the efficiency, use of a bioreactor is desirable. Using a small bioreactor (4 to 10 liters), the author has produced over 4,000 to 10,000 plantlets within 1 to 2 months. The bioreactor system allows the induction of somatic embryos from vegetative cells which then leads to the production of artificial seeds (Redenbaugh et al., 1987).¹²

### 3.4 Bioreactors—Hardware Configuration

The configuration of bioreactors most frequently used for plant cell, tissue, and organ cultures is fundamentally the same as that used for microbial or animal cell cultures. However, in plants, the cells, tissues, and organs are all susceptible to mechanical stresses by medium aeration and agitation. At times, the production of both cells mass and metabolites is repressed severely and the bioreactor must therefore have the characteristics of low shear stresses and efficient oxygen supply. For these reasons, different bioreactors (Fig. 18) have been investigated in order to select the most suitable design. Wagner and Vogelmann (1977)¹³ have studied the comparison of different types of bioreactors for the yield and productivity of cell mass and anthraquinone (Fig. 19). Among different types of bioreactors, the yield of anthraquinones in the air-lift bioreactor was about double that found in those bioreactors with flat blade turbine impellers, perforated disk impellers, or draft tube bioreactors with Kaplan turbine impellers. It was also about 30% higher than that of a shake flask culture. Thus, the configuration of the bioreactor is very important and development efforts are underway for both bench scale and pilot scale bioreactors.

**Aeration-Agitation Bioreactor.** This type of bioreactor (Fig. 20) is popular and is fundamentally the same as that used with microbial cultures. For small scale experiments, the aeration-agitation type bioreactors is widely used. However, when the culture volume is increased, many problems arise. The following are some of the scale-up problems in large aeration-agitation bioreactors: (i) increasing mechanical stresses by impeller agitation and (ii) increasing foaming and adhesion of cells on the inner surface of the bioreactor. Despite these problems, a large scale pilot bioreactor (volume 20 kl) was constructed. It successfully produced both cell mass and metabolites. This bioreactor is therefore the most important type for bioreactor systems.
Air Driven Bioreactors. The simplest design is the air-driven bioreactor equipped with sparger at the bottom of the vessel. It is widely used for plant cell, tissue, and organ cultures. In cases where the cells grow rapidly and the cell mass occupies 40–60% of the reactor volume, the flow characteristics become non-Newtonian and the culture medium can no longer be agitated by simple aeration.

Rotating Drum Bioreactor. The rotating drum bioreactor (Fig. 21) turns on rollers and the oxygen supply mechanism is entirely different from either the mechanically agitated or the air-lift bioreactor. Tanaka et al., (1983), reported that the oxygen transfer coefficient is affected by a change of airflow rate under all rotational speeds (Fig. 22). This characteristic is suitable not only for the growth of plant cell, tissue, and organs but also for the production of metabolites under high viscosity and high density cultures. It is superior to the cultures using either mechanically agitated or air-lift bioreactors since the cultures are supplied ample oxygen and are only weakly stressed. Recently a 1 kl bioreactor of this type was constructed and used for a pilot scale experiment (Tanaka 1987).
Figure 20. Ninety-five liter automated bioreactor for plant cell, tissue and organ cultures. (Photo courtesy of K. F. Engineering Co., Ltd., Tokyo).

Figure 21. Schematic diagram of the rotating drum bioreactor (Tanaka, H., et al., 1983)
Spin Filter Bioreactor. This type of bioreactor (Styer, 1985) is equipped with a filter driven by a magnetic coupling in the stir plate (Fig. 26). This spinning filter operates as a medium agitator without generating shear stress and also serves as an excellent filter for the removal of the medium from the bioreactor without the cells plugging it. The spin filter bioreactor will be most suitable for the continuous culture of plant cells. When a conventional bioreactor was used and the feeding rate of the medium was increased, the cell density was decreased because of washout. However, when a spin filter bioreactor was used, the cell density was maintained constant and half of the spent medium was effectively removed through the spin filter.

Gaseous Phase Bioreactor. As shown in Fig. 24, this type of bioreactor is equipped with filters on which the culture is supported and with a shower nozzle for spraying on the medium (Ushiyama et al., 1984; Ushiyama, 1988). Seed cultures are inoculated on the filters and the medium is supplied to the culture by spraying from a shower nozzle. The drained medium is collected on the bottom of the bioreactor. This type of bioreactor is excellent for plant cell, tissue, and organ cultures because there is no mechanical agitation (e.g., driven impeller, aerator) and, therefore, the growth rate and the secondary metabolite production are enhanced.

Light Introducing Bioreactor. Plants are susceptible to light irradiation and as a consequence various metabolic and/or physiological changes are generated. Some important reactions are: (i) photosynthesis, (ii) activation of specific enzymes such as phenylalanine ammonia lyase (PAL) and to induce the production of flavonoids or anthodyanins, (iii) photomorphogenesis such as development of leaves. For these reactions, the
introduction of light into the bioreactor is required. Inoue (1984)[19] reported a bioreactor equipped with transparent pipes. The light was emitted from the surface of the pipe into the bioreactor. Ikeda (1985)[20] reported an air-lift bioreactor equipped with a photo introducing draft tube (Fig. 25). The draft tube was constructed as an airtight tube which consisted of a transparent inner and outer tube. Within the center of the draft tube was a light introducing optical fiber. The light source was a sunlight collector system which operated automatically by computer control and the collected light was introduced into the bioreactor through the optical fibers. Introduction of light into the bioreactor will become an important technique for the production of specific plant metabolites.

Figure 23. The spin filter bioreactor (Styer, 1985).
Figure 24. Gas phase bioreactor (Ushiyama, et al., 1984).
Figure 25. Photo introducing bioreactor (Ikeda, 1985).
Gas Permeable Membrane Aerator Bioreactor. This type of bioreactor has not yet been fully developed. Nevertheless, some information is available. For example, one bioreactor is equipped with an aerator composed of fine tubes made of polycarbonate, polypropylene, silicone gum, etc. This type of bioreactor should be valuable for immobilized plant cell cultures.

3.5 Bioreactor Size

For industrial production of secondary metabolites, large scale bioreactor systems (sometime over 100 kI) will be required. The 75 kI and 20 kI bioreactor systems used for pilot scale experimentation are at present the largest in the world. They are at the DIVERSA Gesellschaft für Bio- und Verfahrenstechnik mbH in Germany and Nitto Denko Co., Ltd. in Japan. When there is a limited demand for a particular metabolite (e.g., a pigment), the production of commercial quantities can be done in the pilot scale bioreactor. Shikonin is produced this way. In 1983, Mitui Petrochemical Industries became the first to commercially produce a plant metabolite by using a 750 l bioreactor. For routine experiments, the smaller bioreactors of vessel volume 1 to 100 liters are more widely used. Small bioreactors with volumes from 1 to 20 liters are used commercially for the production of clonal plants. These small bioreactors are valuable for the rapid propagation of large numbers of clonal plantlets. Through asexual embryogenesis, 10,000 to 1,000,000 embryos can be produced per liter and these embryos are then grown to plantlets. Using 2 to 10 l bioreactors, it is also possible to produce 5,000 to 10,000 plantlets from plant tissue, which can then be transplanted directly into soil.

3.6 Culture Period

The growth of plant cells, tissues, and organs is much slower than microbial organisms. The most rapid growth cell line reported in scientific journals is the bright yellow *Nicotiana tabacum* cv. (Noguchi et al., 1987). The doubling time of this cell strain was 15 h, and the duration to maximum growth was 80 h (3.3 days) when cultured in a 20 kI pilot scale bioreactor. In general, the growth of the cells of herbaceous annual plants is rapid and their doubling time is usually about 1 to 3 days (duration to maximum growth was 10 to 20 days), and that of woody plants or differentiated organs is slow (doubling time is about 2 to 10 days and the culture period is about 20 to 100 days).
3.7 Aeration and Agitation

The oxygen requirement of plant cells is quite low compared to microorganisms. Kato et al. (1975)\textsuperscript{22} have examined the effect of $k_La$ on biomass production (Fig. 26a). They observed that the volumetric oxygen transfer coefficient, $k_La$ was constant after 10 h and the final biomass concentration became constant at 0.43 g cell dry weight/g sucrose. When $k_La$ was set under 10 h, cell yield became dependent on $k_La$ values. The effect of agitation speed on final cell mass concentration was also analyzed by Kato et al. (1975)\textsuperscript{22} using a 15 l bioreactor (Fig. 26b). At lower agitation speeds (less than 150 rpm), cell mass concentration became constant. However, when the agitation speed exceeded 150 rpm, the cultures became bulky and started to foam profusely. An agitation speed of either 50 or 100 rpm seemed to be optimal for production of cell mass and also for avoiding the culture problems.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure26.png}
\caption{Effect of initial oxygen transfer coefficient and agitation by turbine impeller on cell growth (Kato, 1975).}
\end{figure}
3.8 Microbial Contamination

According to Manfredini et al. (1982), the most frequent factors causing microbial contamination include: (i) construction materials, (ii) seals and valves, (iii) complexity of the plant, (iv) operator error, (v) instrumentation failure, (vi) process air, (vii) transfer and feed lines, (viii) contamination from vegetative preculture, (ix) critical medium composition, (x) inadequate procedures. As the culture periods of plant cell, tissues, and organs are usually quite long, (particularly for continuous cultures), special designs and operations are necessary to avoid microbial contamination. For example, Hasimoto et al. (1982) reduced contamination in their 20 kl bioreactor by using three air filters in a series; the third filter was a membrane of uniform pore size of 0.4 μm. The design specified aseptic seals in the agitator shaft and exit pipelines for sterilized air after steam sterilization.

3.9 Characteristics

The special characteristics of plant cells that tend to hamper large scale cultivation of the cells are described below.

Bubbling and Adhesion of Cells to the Inner Surface of the Bioreactor. Plant cell cultivation is usually performed by bubbling and agitation which cause foaming and adhesion of the cells to the surface of a bioreactor. Because of this phenomenon, cell growth is inhibited. The authors (Takayama et al., 1977) have examined the possible causes and concluded that the adhesion of cells appeared to be the result of gel formation from pectin and calcium. By reducing the concentration of CaCl₂·2H₂O in the medium, the foaming and the number of cells that adhered to the walls was decreased markedly. The cells became easily removable from the inside wall of the fermenter and were returned to the medium. Cell destruction was measured by A660 values which also depend on the CaCl₂·2H₂O levels. A lower level of CaCl₂·2H₂O in the medium markedly inhibits cell destruction. These observations are particularly pertinent when large-scale cultivation is being considered.

Cell Morphology and Specific Gravity. According to Tanaka (1982), plant cells have a tendency to grow in aggregates of different sizes. The size distribution of cell aggregates is different from one plant species to another (Tanaka, 1982). Specific gravity of these cells ranges from 1.002 to 1.028. If the diameter of the cell aggregate is less than 1 to 2 mm, the cells can be suspended and do not sink to the bottom of the bioreactor (Tanaka, 1982), but, when the specific gravity of the cell is greater than 1.03, the
diameter of the aggregate becomes 0.5 to 1.0 cm and the cells sink to the bottom of the bioreactor and cannot be suspended. When the agitation is increased, the size of the cell aggregate becomes smaller (Tanaka, 1981), but the growth of the cell is repressed. In order to separate the cells from the aggregate, the amount of calcium is decreased to suppress the gel formation of pectin which plays an important role in the cell, e.g., cementing plant cells, but has little effect on cell separation (Takayama, 1977).

Viscosity, Fluidity, and Oxygen Supply. When plant cells grow well, they can occupy 40 to 60% of the whole culture volume, and the apparent viscosity becomes very high. Tanaka (1982) examined the relationship between apparent viscosity and concentration of solids in suspension, and concluded that when the cell density exceeds 10 g/l, the slope of the apparent viscosity increases rapidly, and when cell density reaches 30 g/l, the culture medium becomes difficult to agitate and supply with oxygen.

Figure 27. Relationship between apparent viscosity and concentration of cells and pseudocells in culture media (Tanaka, 1982). (□) C. Roseus, (○) C. Tricuspidata B., (△) N. tabacum L., (+) granulated sugar.
3.10 Manipulation

**Large Scale Batch Culture.** Batch culture systems are in use worldwide and many experimental results have been reported using 10 l to 20 kl bioreactors. Noguchi et al. (1987)\[21\] have examined the growth of tobacco BY-2 cells using a 20 kl aeration-agitation bioreactor with 15 kl medium. The medium used was Murashige and Skoog's inorganic nutrients with three times the normal amount of phosphate and 3% sucrose, incubated at 28°C and aerated at 0.3 vvm. The results revealed that the highest growth rate was observed from the incubation time of 45 to 70 hours with a doubling time of about 15 hours, which was almost the same as the growth in flask cultures. Ushiyama et al. (1986)\[8\] examined the growth of Panax ginseng root cultures in 30 l, 2 kl, and 20 kl aeration-agitation bioreactors. The productivity of the cultures in 2 kl and 20 kl bioreactors was 700 and 500 mg/l/day in dry weight, respectively. Building upon this basic research, large scale batch culture techniques have been developed for the industrial production of cell mass. However, culture conditions suitable for cell mass production are not always suitable for secondary metabolite production. Accordingly, in order to produce both cell mass and metabolites efficiently, two-stage culture techniques have been adopted. This technique uses two batch bioreactors and was first reported by Noguchi et al. (1987)\[21\] for the production of low nitrogen content tobacco cells. In the 1980's, this technique was widely used for secondary metabolite production such as shikonin (Fujita et al.),\[27\] rosmarinic acid (Ulbrich, 1985)\[27\] and digoxin production (Reinhard, 1980)\[28\].

For shikonin production by *Lithospermum erythrorhizon*, two-stage cell culture was used (see Fig. 28). The first stage culture was grown in a MG-5 medium which was suitable for cell mass production. It was then transferred to 2nd-stage culture where it was grown in an M-9 medium, modified by a higher Cu^{2+} content and a decreased salt content.

**Large Scale Continuous Culture.** The growth rate of plant cells is usually low compared to that of microbial organisms. In order to enhance the productivity of cell mass and metabolites, continuous culture methods should be employed (Wilson, 1978)\[29\] Fig. 29a). In the research laboratories of Japan Tobacco and Salt Co., a pilot plant (1500 l) and an industrial plant system (20 kl) have been used for developing continuous culture techniques (Hashimoto, et al., 1982)\[24\] Azechi et al., 1983)\[30]\). A 20 kl bioreactor having a working volume of 6.34 kl, was used for the experiment (Fig. 29b) and ran for 66 days of continuous operation. The conditions were: aeration rate, 0.35–0.47 vvm; agitation speed, 27.5–35 rpm; dilution rate, 0.28–0.38
days. In this experiment, the residual sugar content was an important index of the operation and, at steady state, its value was maintained above 5 g/l. Other control parameters such as aeration, agitation, and dilution rates were changed gradually. The success of this experiment will soon lead to the establishment of long term industrial continuous culture systems for secondary metabolite production.

**Immobilized Culture.** Immobilization of plant cells was first reported by Brodelius et al. in 1979,[31] and since then many reports have been published. Unfortunately, an immobilized cell culture technique has not yet been established as an industrial process for secondary metabolite production. However, this technique has many excellent features and should be the subject of future development research.

![Figure 28. Two stage culture methods (Fujita, 1984).](image-url)
Figure 29. Continuous culture system. (A) Small glass vessel (Wilson, et al., 1976). (B) Pilot plant for continuous cultures with 20 kL bioreactor (Azechi, et al., 1983).
3.11 Scale-up Problems

Scale-up techniques for plant cell cultures are not well understood. Some attempts have been made using tobacco cells and applying essentially the same parameters as those for the scale-up of microbial cultures (Azachi, 1985). The results showed that $k_a$ values are useful as scale-up parameters, however, the situation for secondary metabolite production is quite different. The productivity of the metabolites decreased as the culture volume increased. An example of this is the productivity of the indole alkaloid, a serpentine which declined significantly as the culture volume increased from 0.1 to 80 l (Fowler, 1987). Possible reasons for the loss of product on scale-up are the following: (i) altered and inadequate mixing of the nutrient and cells at the high reactor volumes and (ii) lowered dissolved oxygen level (Breuling et al, 1985). Fujita and Tabata (1987) used the scale-up of suspension cultures of *Lithospermum erythrorhizon* cells for their ability to produce shikonins as the criterion for comparing the aeration-agitation type bioreactor with a modified paddle impeller and the rotary drum.
type bioreactor. When the aeration-agitation type bioreactor was scaled up to a volume of 1000 l, the shikonin productivity decreased, but when the rotary drum bioreactor was scaled up to 1000 l, there was no decrease in the yield of shikonins (Fig. 31). Thus, in an industrial pilot plant for secondary metabolite production, mild agitation and oxygen supply will be important variables.

![Figure 31](image)

**Figure 31.** Scale up of suspension cultures for Lithospermum erythrorhizon cells. (Fujita and Tabata, 1987).

### 3.12 Bioprocess Measurement and Control

**Bioprocess Measurement and Control in Large Scale Culture.**

Measurement and control systems used in the bioreactor for plants are essentially the same as those for microbial or animal cell cultures, but, in special cases, where the mineral components influence the productivity of secondary metabolites, the kind of salts used for the electrode must be taken into consideration.

**Mass balance.** The mass balance equation (Eq. 4) is generally used for heterotrophic organisms. Pareilleux and Chaubet (1980)[36] have applied the equation to apple cell cultures:

\[
\frac{1}{X} \frac{dS}{dt} = \frac{1}{Y_G} \cdot (\mu + m)
\]
where

\[
\frac{1}{X} \cdot \frac{dS}{dt} = \text{specific rate of sugar consumption (g sugar / g cell dry weight · h)}
\]

\[
\mu = \text{specific growth rate (h)}
\]

\[
Y_G = \text{maximum growth yield (g cell weight / g sugar)}
\]

\[
m = \text{maintenance coefficient for sugar (g sugar / g cell dry weight / h)}
\]

From the equation, \( m \) and \( Y_G \) can be estimated from a plot of \( X \cdot (dS/dt) \) against \( \mu \). The values observed for \( Y_G \) and \( m \) are in good agreement with those reported previously by Kato and Nagai (1979) [37] calculated from tobacco cell cultures; i.e., \( m \) values were smaller and \( Y_G \) values were higher compared to the values reported for many microorganisms.

**Measurement and Mathematical Fitting of Cell Growth.** Richards (1960) used a generalized logistic curve for the mathematical fitting of the growth curve of plants. The equation is:

\[
\text{Eq. (5)} \quad \ln \frac{A-W}{W-B} = f(t)
\]

where

\[
A = \text{asympotic value of dry weight}
\]

\[
B = \text{inoculated value of dry weight}
\]

\[
t = \text{days culture in vitro}
\]

\[
W = \text{dry weight at any instant } t \text{ (days or hours)}
\]

\[
f(t) = \text{suitable function of time } t \text{ (days or hours)}
\]

By eliminating the natural logarithm

\[
\text{Eq. (6)} \quad W = B + (A-B)/[1+e^{f(t)}]
\]

Differentiating with respect to \( t \):
The above equation is the growth rate. To calculate the specific growth rate, \( u \), divide by the dry weight.

\[
\begin{align*}
\text{Eq. (7)} & \quad \frac{dW}{dt} = - \frac{(A - W)(W - B)}{A - B} f'(t) \\
\text{Eq. (8)} & \quad \mu = \frac{1}{W} \frac{dW}{dt} \\
\text{Doubling time, (T) becomes:} & \\
\text{Eq. (9)} & \quad T = \frac{\ln(2)}{\mu}
\end{align*}
\]

References (Section 3)


29. Wilson, G., Growth and product formation in large scale and continuous culture systems, *Frontiers of Plant Tissue Culture*, pp. 169–177, University of Calgary, Canada (1978)


