

Chapter 14

Strategies for Monitoring and Modeling the Growth of Hairy Root Cultures: An In Silico Perspective



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Abstract Hairy roots have been identified as a good source of secondary metabolites in plants. These secondary metabolites in the genera of phytochemicals have been used by humans since long in the form of drugs, flavors, colors, and others. Thereby, large-scale culture of hairy roots, its management, and production have been conferred as most important and critical steps at industrial scale. Conversely, culture of hairy roots in bioreactors at industrial scale has proven to be a tedious job and requires continuous monitoring and precise control of the system. These challenges for hairy roots owe to their heterogeneous nature. Conventional methods for monitoring of such cultures have failed to work well within this system. So, indirect methods are being used for continuous monitoring of growth and metabolite content in hairy roots. Efficiency and efficacy of these indirect methods depend largely upon models of hairy root growth, product synthesis, and substrate utilization. Several mathematical and computational models have been developed to explain hairy root growth. Some of these models are complex mathematical equations which are based on physical principles, while others are computational models derived from empirical data. This chapter intends to outline and explain some of the prominent models for hairy root growth and their mode and mechanism of action in large-scale bioreactors.

Keywords Artificial neural network · Genetic algorithm · Hairy root culture · Hidden Markov model · Image analysis · Mathematical model · Metabolic flux analysis

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14.1 Introduction

Hairy roots are plant disease syndrome caused by the infection of soil-borne bacterium *Agrobacterium rhizogenes* to the higher plants. Upon infection *A. rhizogenes* transfers a DNA segment (T-DNA) from its root-inducing (Ri) plasmid into the genome of the host plant. Set of genes, carried by T-DNA segment, codes for the enzymes which interfere into the auxin and cytokine in the biosynthesis of the host (Chandra 2012; Mehrotra et al. 2015; Srivastava et al. 2016). Due to this reason, the hormonal imbalance at the wounded site occurs that causes the rapid growth of hairy rootlike mass at the wounded sites. These are called hairy roots and are prominent disease syndrome of higher plants (Georgiev et al. 2012). The hairy roots are characterized by high growth rate, genetic stability, and growth in hormone-free media. These roots are very much similar to the native roots of the host plant in terms of their capacity to produce similar or much higher amount of secondary metabolites that are normally produced by the plant (Pistelli et al. 2010). In laboratories, hairy roots are cultured to explore secondary metabolite synthesis both in terms of their production for commercial purposes and to investigate their biochemical and molecular aspects. Hairy root cultures have proven their worth for the production of commercially valuable secondary metabolites. This property of hairy roots is of particular interest for researchers who strive for large-scale production of these metabolites. Secondary metabolites are the chemicals produced by plants for which no role has been found in growth, photosynthesis, reproduction, or other primary functions. Humans use some of these compounds as medicines, flavorings, fragrance, recreational drugs, biopesticides, nutrients, cosmetic additives, etc. The procurement of these phytochemicals at large scale from plants requires vast agricultural land, time and labour. Further, their chemical synthesis is a costly and labor-intensive affair. Therefore, the constant use, ever-increasing demand, and less availability of these important phytochemicals from natural sources are the driving efforts to develop new ways to optimize their alternative production. In this reference, hairy root-based production of plant-derived metabolites needs scientific consideration. Another commercially important aspect of hairy root is their ability to produce proteins. Although there are some problems associated with production of foreign proteins by hairy roots like low accumulation levels, instability of proteins, etc., hairy roots have great potential for large-scale production of proteins (Doran 2006). Furthermore, hairy root cultures are known for their use in value-added applications like phyto-/rhizoremediation of toxic compounds, biotransformation of exo-/endogenously supplemented substrates into commercially more valuable compounds, etc. (Mehrotra and Srivastava 2017; Srivastava et al. 2017).

To sum up, hairy root cultures have gained popularity as one of the most suitable biological systems to fulfill various biotechnological objectives. For this purpose, the establishment and maintenance of the culture system is a prerequisite. Further, before using any hairy root culture system, sometimes there is a need to know about their biological behavior and their responses to various environmental factors as these factors determine their overall growth and productivity. Practically, in

biological systems it is troublesome and tedious to optimize all the factors every time before using a system for any purpose as biological behaviors are nonlinear and nondeterministic. For this question, the use of modeling of biological systems to investigate their growth patterns, responses to their physical and chemical environment, production potential, etc. can provide an answer. The upcoming text provides condensed information about modeling of biological system and their applications with particular reference to hairy root cultures.

14.2 Modeling of Biological Systems

Advances in various fields of biology and information technology have produced huge amounts of data. Accumulation of this data is day to day increasing and is in continuous need of interpretation and investigation in order to understand the behavior of biological systems. These advances are changing the way biological research, development, and applications are conducted. Modeling is the human activity which includes representation, manipulation, and communication with real-world life objects. A model can be defined as a portrayal of a system (comprised of many integral parts) in terms of its constitutive parts and their association/interactions, where the portrayal itself is decodable or interpretable by humans. Biological processes are nonlinear and complex because of their collective behavior and changes in various phases of development. They depend upon different external and internal factors (Gago et al. 2009). The challenge in modeling any biological process is to find a model which is accurate and able to provide deep insight of the process. Because of the complexity of biological data, simple algorithms or mathematical equations cannot be used to describe the process (Osama et al. 2015). Deterministic models of biological process have been developed based on physical interactions. These models are helpful in providing underlying information of the process. However, due to complexity of biological systems, these models are difficult to develop and are very complex to interpret and solve. Modeling the biological processes requires accounting for action and feedback involving a wide range of spatial and temporal scale. The upcoming text provides an informative description upon various approaches for modeling in hairy root cultures to explore their growth and production phenomenon. Some models of hairy root growth in bioreactors have been summarized in Table 14.1.

14.3 Statistical and Mathematical Models for Hairy Root Growth

Statistical designs can be used to model relationships of different factors on hairy root growth. These designs are simple and easy but require some prior data. Bhadra and Shanks (1995) used statistical design to model the effect of inoculum conditions

Table 14.1 Summary of reported models for the hairy root growth

S. no.	Model	Hairy roots	Effects of model	References
1.	Box-Behnken design (BBD)	<i>Isatis tinctoria</i>	Secondary metabolites (rutin, neohesperidin, buddleoside, liquiritigenin, quercetin, isorhamnetin, kaempferol, and isoliquiritigenin)	Gai et al. (2015)
2.	Agent-based modeling (ABM) approaches	<i>Beta vulgaris</i>	Total root length, branching point distribution, segment distribution, and secondary metabolite accumulation	Lenk et al. (2014)
3.	Artificial neural network in combination with hidden Markov model	<i>Rauwolfia serpentina</i>	Overall productivity of a bioprocess	Mehrotra et al. (2013)
4.	Artificial neural network-based model	<i>Artemisia annua</i>	Effect of different reactor parameters on hairy root biomass	Osama et al. (2013)
5.	Artificial neural network (ANN)	<i>Glycyrrhiza glabra</i>	Prediction of optimal culture conditions for maximum hairy root biomass yield	Prakash et al. (2010)
6.	Mathematical model	<i>Artemisia annua</i>	On and off cycle in a nutrient mist reactor	Ranjan et al. (2009)
7.	Multi-scale mathematical model	<i>Ophiorrhiza mungos</i> Linn.	Temporal evolution of biomass increase and nutrient uptake	Bastian et al. (2008)
8.	Structured nutritional model	<i>Catharanthus roseus</i> , <i>Daucus carota</i>	Secondary metabolites (nitrogenous compounds and storage carbohydrates, recombinant protein)	Cloutier et al. (2008)
9.	Feed-forward back propagation neural network-based model	<i>Glycyrrhiza glabra</i>	Optimum culture condition on biomass growth	Mehrotra et al. (2008)
10.	Population-based model	<i>Helianthus annuus</i>	Biomass increase based on age distribution of cells and branching	Han et al. (2004)
11.	Kinetic model for pigment associated with root growth	<i>Beta vulgaris</i>	Kinetic behavior of root and pigmentation based on hairy root growth	Kino-oka et al. (1995)
12.	Aerosol model	<i>Artemisia annua</i>	Deposition of mist droplets on root hairs in a nutrient mist reactor	Wyslouzil et al. (1997)
13.	Branching number and age	<i>Tagetes erecta</i>		Kim et al. (1995)

(continued)

Table 14.1 (continued)

S. no.	Model	Hairy roots	Effects of model	References
	distribution-based model		Specific growth rate of hairy root based on branching dynamics	
14.	Image analysis	<i>Brassica napus</i> , <i>Brassica campestris</i>	Assessment of phenotypic effects of expressing foreign genes in plant root systems	Coles et al. (1991)
15.	Comprehensive model based on conductivity	<i>Coffea arabica</i> , <i>Nicotiana tabacum</i> , <i>Withania somnifera</i> , <i>Catharanthus roseus</i>	Dry weight of hairy root based on conductivity	Taya et al. (1989a)
16.	Kinetic model for branching	<i>Daucus carota</i> <i>Armoracia</i> <i>lapathifolia</i> <i>Cassia torosa</i> <i>Ipomoea aquatica</i>	Increase in hairy root biomass	Taya et al. (1989b)

on growth of hairy roots of *Catharanthus roseus*. In this study, a two-level factorial design was used to study the effect of a number of root tips inoculated, the length of inoculated root tips, and the initial volume of media. Experimental and statistical analysis demonstrated that hairy root growth is highly influenced by the inoculum conditions particularly with the length of root tips which were the dominant variable without any clonal variability. Statistical designs have also been extensively used in media optimization. Growth media composition plays most an important role for both growth and productivity in any culture practice. Thus, during initiation and maintenance of culture, variables of chemical and physical conditions as well play a dominant role. Additionally, the biological condition of culture initiating material (explant) also plays a definite role in growth, development, and productivity. Therefore, optimization of these culture conditions is a prerequisite. Traditionally, optimization of media in biological analyses has been carried out by monitoring the influence of one factor at a time. This technique is called one-variable-at-a-time. However, the main disadvantage of this method is that it does not include any interactive effects among the variables studied. Further, it requires a large number of experimental set, labor and cost inputs, and finally time consumption (Bezerra et al. 2008). To avoid all these limitations, statistical methods for optimization of various culture conditions have been introduced in various culture systems including hairy root cultures (Toivonen et al. 1991; Srivastava and Srivastava 2012). Among initial studies, the effect of sucrose, phosphate, nitrate, and ammonia concentrations on growth and indole alkaloid production of *C. roseus* hairy root cultures was investigated with the help of statistical experimental designs and linear regression analysis (Toivonen et al. 1991). Interestingly, a contradictory effect of these nutrients on growth and indole alkaloid production in *Catharanthus* hairy roots was found. Statistical medium optimization for enhanced azadirachtin production from

the hairy root cultures of *Azadirachta* is one of the most cited examples of the use of statistical methods in media optimization (Srivastava and Srivastava 2012). Plackett-Burman experimental design protocol was used to identify dominating medium components and their concentrations to support high root biomass production and azadirachtin accumulation in hairy roots. The overall exercise has resulted in increased azadirachtin production by 68% in *Azadirachta indica* hairy roots. RSM are efficient tools for optimization, and an increase in productivity by more than 200% can be achieved. In another study, medium optimization for hairy root cultures of *Stizolobium hassjoo* producing secondary metabolites was studied through statistical experimental design (Sung and Huang 2000). The increased production of L-DOPA from hairy roots by 280% was obtained by optimizing medium components using steepest ascent method with central composite design. Also, the study reported 18% increase in the biomass of *Stizolobium hassjoo* hairy roots from the basal media.

Response surface methodology (RSM) has evolved as the most popular optimization method having versatile applicability in various disciplines. RSM is a well-known, dynamic, and efficient mathematical approach which comprises of statistical experimental designs and multiple regression analysis which are the best combination for the formulation of constrained equations (Bezerra et al. 2008). RSM has often applied for the optimization of the fermentation as well as hairy root cultivation (Amdoun et al. 2010; Latha et al. 2017; Singh et al. 2017; Adebo et al. 2018).

RSM is a combination of mathematical and statistical techniques used for modeling of process based on empirical parameters. This method optimizes the response (output variable) which is dependent upon several independent variables (input variable). RSM requires a careful design of experiment; most commonly central composite design or Box-Behnken design is used. In this technique a second-order mathematical model is developed to relate response and independent variables. The model is then differentiated to find the global maxima or minima in order to optimize response (Amdoun et al. 2010). RSM allows the researcher to study the interactive effect between the independent variables. RSM has also been used to optimize the culture medium composition for the growth of elicited *Datura stramonium* L. hairy roots to improve the production of hyoscyamine (Amdoun et al. 2010). In B5 medium the content of nitrate, calcium, and sucrose was optimized to get the best hyoscyamine production. In continuation of the study, the use of the RSM was also made in biological factors, like plant material, to establish a predictive model with the planning of experiments, analysis of the model, and interpretation of the accuracy of the model. Also, the effect of nitrogen, phosphorus, potassium, calcium, and magnesium ions on production of tropane alkaloids from *Datura stramonium* hairy roots was investigated with the help of RSM. The model was developed to study the effect of ions on production of hyoscyamine from *Datura stramonium* hairy roots with elicitation and without elicitation (Amdoun et al. 2009, 2010).

In a recent study, *A. rhizogenes*-mediated hairy root cultures of *Portulaca oleracea* were established for which Box-Behnken model of response surface methodology (RSM) was employed to optimize B5 medium for the growth and noradrenaline production. Upon experimental validation, the optimal conditions for

growth and metabolite production predicted by RSM were confirmed as appropriate for the enhancement of overall productivity (Ghorbani et al. 2015). Further, in a similar study, one-factor model of RSM was utilized to formulate L-arginine amino acid levels along with bacterial strains (ATCC 15834, C58C1, and R1000), type of explant (leaf and stem), and co-cultivation medium (B5 and MS) as three different variables for hairy root induction in *Rubia tinctorum* (Ghorbani et al. 2014). According to the results, L-arginine concentration of 1.00 mM, bacterial strain C58C1, leaf explant, and B5 medium were found optimal for best results. These optimal conditions predicted by RAS were validated and confirmed experimentally to enhance hairy root induction and its implementation for increased metabolite production.

In this context, growth monitoring of hairy roots in liquid medium is a major point of concern. Basically, the growth monitoring in liquid medium is required during large-scale cultures of hairy roots in bioreactors for various purposes. In a culture vessel, during running culture one can visually observe the growth and distribution of hairy root tissue throughout the vessel. However, it is not possible to measure the biomass accurately like this. Several mathematical models have been developed for the estimation of biomass and related metabolite accumulation in hairy root cultures keeping in mind the bioreactor type and culture vessel configuration. The complex interplay of variables like dissolved O_2 , temperature, aeration and agitation rates, pH, etc. is monitored, and values are inserted to mathematical models. These models interpret the values and predict the results very near to accuracy in a very short time. The synergistic and individual roles of various variables are thus defined, and in this way growth of subjected root is monitored and maintained throughout culture duration. This ultimately leads to desired productivity. An online monitoring of growth characteristics of hairy root cultures was done by the measurement of conductivity in the bioreactor system (Taya et al. 1989a). A comprehensive model was developed based on conductivity measurements to assess the biomass concentration of hairy root cultures of *Coffea arabica*, *Nicotiana tabacum*, *Withania somnifera*, and *Catharanthus roseus*. A linear relationship between dry cell mass and conductivity for all the root cultures was observed. This method provided an effective means of in situ monitoring of hairy growth in the culture. Later this method was used to determine the biomass concentration of horseradish and carrot in stirred tank and airlift loop bioreactors (Taya et al. 1989c). During the cultivation of high-density biomass, the volume of liquid media reduces with the growth. This change in media volume was considered as a parameter for estimation of biomass growth. Jung et al. (1998) used this method for estimation of biomass of *Catharanthus roseus* hairy root in a 2 liter bubble column bioreactor. It was observed that this method was more accurate in biomass prediction than the conventional method using electrical conductivity as the only parameter. However, in another study, electrical conductivity (EC) in the media along with kinetics of changes in ion concentrations and sugar was monitored to understand the relationship between growth, ginsenoside production, and nutrient partitioning with the help of gaseous composition gradient in terms of O_2 , CO_2 , and ethylene content (Jeong et al. 2006). In another study, a 3 L nutrient trickling reactor was operated on the

basis of online monitoring of conductivity, pH, and dissolved oxygen. An enhanced production of L-DOPA was observed from hairy root culture of *Stizolobium hassjoo* (Huang et al. 2004). In an earlier study, Wyslouzil et al. (1997) developed an aerosol model for deposition of mist droplets on root hairs in nutrient mist reactor. The *Artemisia annua* hairy root bed was assumed as a fibrous filters, and a model was prepared for mist deposition on the single root fiber. In this study, the deposition of mist across a packed bed of roots was modelled as a function of droplet size, bed length, and gas flow rate. The predictions of the aerosol deposition model were validated with experimental measurements which were found similar.

In nutrient mist reactor, the time of mist on and off cycle is very important. If on cycle is long, it results in accumulation of media on hairy roots causing a gas-phase nutrient deficiency. While during long off cycle, roots can be starved of liquid-phase nutrients. A mathematical model for the mist on and off cycle was prepared by Ranjan et al. (2009), for maximum root density and root growth in nutrient mist reactor. If the mist flow rate in the on cycle is low and the rate of drainage of the media from the bed is equal to the rate of mist deposition, then the reactor could be run in a continuous on cycle. In nutrient mist reactors, to study the kinetic growth of hairy roots, a discrete model was developed (Ranjan et al. 2015). The elongation rate is modeled as exponential growth with the growth coefficient being dependent on mass transfer coefficient, nutrient concentration difference, and distribution of nutrients in growth and sustenance requirements. The experimentally validated results have shown that the primary root growth is reduced by one-fifth of its initial growth rate due to the branching process, and the growth of new branches is significantly faster than its primary root growth due to internal transport of nutrients. Bastian et al. (2008) used a multi-scale approach to simulate hairy root growth. They treated root bulk as a macroscopic porous filter of varying porosity, and all processes were defined in its continuum. The growth was assumed to depend upon nutrient concentration in the medium and inside the root. On microscopic scale the structure of root affects the flow and transport process of nutrients around the root network.

Attempts have been made to model the branching pattern in hairy roots. Hairy roots generally grow by elongation of nodes. New nodes develop and on elongation they form new branches. A kinetic model of branching in hairy roots was developed and simulated for different root cultures in shake flask (Taya et al. 1989b). The model was found to fit the experimental results and was used to estimate different kinetic parameters of hairy roots. Kim et al. (1995) developed a mathematical model for describing branching patterns in hairy roots. This model was then combined with age distribution balance to give a model of age distribution in root culture. Similarly, Han et al. (2004) modeled hairy root growth based on population balance approach. The model proposed that growth of hairy roots depends on formation of new branches and elongation of existing branches. Although probability of formation of new branch is high at a certain age, some lateral branches can develop over distribution of ages of the parent branch.

The growth of hairy roots can also be monitored by the pigment production. A kinetic model was developed for pigment production associated with growth of red beet hairy roots (Kino-oka et al. 1995). The model was based on concept of

distribution of age of cells in hairy roots. The model was able to describe the kinetic behaviors of growth and pigmentation during hairy root growth.

Mass and oxygen transfer rates have a crucial role in the growth of hairy roots in liquid medium. Different workers have proposed methods to investigate and optimize this event for desired productivity. In a study, a mathematical model is developed that defines the oxygen transfer kinetics in the cultured *Azadirachta indica* hairy root matrix as a case study for offline simulation of process control strategies ensuring non-limiting concentrations of oxygen in the medium throughout the hairy root cultivation period. The unstructured model simulates the effect of oxygen transfer limitation in terms of efficiency factor on specific growth rate of the hairy root biomass. The model is able to predict effectively the onset of oxygen transfer limitation in the inner core of the growing hairy root matrix such that the bulk oxygen concentration can be increased so as to prevent the subsequent inhibition in growth of the hairy root biomass due to oxygen transfer (diffusional) limitation (Palavalli et al. 2012).

14.4 Image Analysis

Image analysis is the procurement of meaningful information from images particularly from digital images with the help of digital image processing techniques. In recent years, many image analysis softwares have been designed for more detailed root morphological and architectural measurements. One example of such software is WinRHIZO root-scanning software (Regent Instruments Inc., Ottawa, ON Canada). This software has the ability of rapid measurement of multiple root parameters such as root length, volume, surface area, diameter, tips, and crossings and has been widely used in research related to plant root growth and responses (Aryal et al. 2015; Kadam et al. 2017). WinRHIZO, however, is a costly software, and several freeware like ROOTEDGE are also available which are highly efficient (Kaspar and Ewing 1997). Image analysis provides an efficient way for noninvasive and nondestructive monitoring of hairy root growth kinetics on the basis of their morphological characteristics (Coles et al. 1991). A manual imaging method “PetriCam” along with an image processing algorithm was initially proposed by Lenk et al. (2012, 2014) to assess the growth performance and secondary metabolite production in *Beta vulgaris* hairy root cultures growing in petri plates. The uniqueness of the method is its ability to take images from the closed petri plates without destroying the culture. However, as the image consists of planner 2D growth pattern, the major limitation of this method is that it does not provide any information regarding the distribution of hairy roots in z-axis. Considering the fact that different HR morphologies result in dissimilar levels of secondary metabolite production, the effect of morphological features on growth and production potential needs proper attention. As two HR clones with a similar biomass but different root architectures could have completely different product yields, it becomes important to investigate their differential production. Image analysis has been successfully adapted by many

researchers for measurement of root length, diameter, and other morphological characteristics (Cai et al. 2015). These workers demonstrate a near to perfect numerical scheme for accurate, detailed, and high-throughput image analysis of plant roots. Involvement of image analysis methods provides better results in terms of accuracy, robustness, and the ability to process root images under high-throughput conditions (Flavel et al. 2017). In context of hairy roots, Berzin et al. in 1997 developed a morphological structured model of *Symphytum officinale* hairy roots using a desktop scanner for image analysis and NIH image program which is a public image processing and analysis program developed by the National Institutes of Health. In another study, nondestructive measurement of the chlorophyll pigment based on color image analysis was done for the assessment of herbal toxicity in photoautotrophic hairy roots of *Ipomoea aquatica* (Ninomiya et al. 2003a). Furthermore, the elongating behavior of *Ipomoea aquatica* hairy roots exposed to external herbicidal stimuli was evaluated by automatic tracing of the root tip point employing computer-aided image analysis (Ninomiya et al. 2003b). Such results advocated that the system developed could be a useful tool for the assessment of herbicidal toxicity in the hairy roots.

Once the images are taken, they are needed to be processed to procure numerical information such as segment length, branching point distribution, metabolite accumulation patterns, etc. In recent years, several efficient open-source and commercial solutions have been reported by several researchers for image processing purposes (Lobet et al. 2011; Clark et al. 2013).

Image analysis can also be used for estimation of secondary metabolite in hairy root cultures (Lenk et al. 2012). Due to accumulation of secondary metabolites, the morphological characteristics of hairy roots change. This change can be analyzed by image analysis to predict secondary metabolite concentration. For the estimation of secondary metabolite, color image analysis is used. Smith et al. (1995) introduced hue-saturation-intensity (HSI) color coordinate system and developed equations for converting the red-blue-green color coordinate system to HSI. They proposed that HSI color coordinate is better for image analysis of hairy roots. Berzin et al. (1999) developed a nondestructive method, based on the analysis of scanned images in HIS color space, for determining local and overall levels of secondary pigment metabolites in hairy root cultures of *Beta vulgaris*. Modified saturation values (saturation divided by dimensionless root diameter) were found to be proportional to pigment concentration. The analysis was carried out manually for each local point of the root, and morphological measurements were performed separately. RHIZOSCAN is semiautomated software for root image analysis. It provides typical measurement analysis, such as root axis length (primary, secondary, total) and comparative plots. It measures root thickness, volume, length, etc. of each lateral of hairy roots and also can be used for estimation of secondary metabolite from scanned images. Berzin et al. (1999) tested this software for characterization of morphology of hairy roots of *Beta vulgaris* and estimation of secondary metabolite concentration. They concluded that RHIZOSCAN is a reliable tool for analysis of root architecture and determination of secondary metabolite in hairy roots.

14.5 Genetic Algorithm (GA)

Economic viability of secondary metabolite production in hairy root cultures depends largely on the kinetic growth model and efficient scale-up in bioreactor designs. A population-based model as genetic algorithm has seen its significant implications in modeling of specifically hairy root cultures owing to its capacity to search solutions in large hyperspace applying schemata theorem and optimizing to its nearest best solutions in the best possible time (Han et al. 2004; Arab et al. 2016). Based on the nature's natural process of evolution, GAs involve a class of computational models lying on the principle of natural selection and survival of the fittest phenomenon (Fogel et al. 1975). GAs have been prominently used as a suitable function in the determination of the optimum concentration of the medium components in hairy root bioreactor design.

Conceptually, genetic algorithms begin with a set of solutions encoded like genes on the chromosomes called population. Motivated by a hope that the new population will be better than the old solutions, individuals from one population are selected on the basis of fitness functions (given more chances to reproduce) and are used to form a new population by the application of natural selection operators. Natural selection is usually applied through stochastic or remainder stochastic sampling techniques. This is followed by the F2 generation (offspring) by the application of recombination operator using one-point or two-point crossover to introduce the genetic diversity in the solutions to the current population. Mutation operation is often implied involving a single flip of bit in binary encoding of individual fitness values which tends to introduce novel solution to the current population (Fig. 14.1). This is repeated to several generations until we move toward to better population of individuals with improved solutions than parent populations (Fogel et al. 1975; Davis 1991). GAs have been continuously applied to solve many search and optimization problems involving non-differentiable, discontinuous, stochastic, or highly nonlinear objective functions, which are normally not well suited for standard optimization algorithms.

GAs in hairy root cultures are based on the fact that age distribution of cell in cell cultures is not uniform and evolves with time, and thus the dependent variable in this population-based model tends to be the biomass at a time t or the number of cells at the time t . A variable X is defined to denote the biomass weight distribution function for hairy roots of age A at culture time. The age of the oldest cell from a branch is taken as the age of the specific branch. The total biomass of hairy root at any time t tends to be a function of $X(t, A)$ where A_{max} is the greatest possible age of hairy root culture during harvesting time. As branching rather than root lengthening accounts for the maximum production of biomass in hairy root cultures, GA model has been specifically designed for branching studies as a function of time and age (Han et al. 2004; Arab et al. 2016). One of the most critical advantages of GA seen is its capacity in handling a large number of data including previous data assumed at each generation in the direction of producing the optimized result.

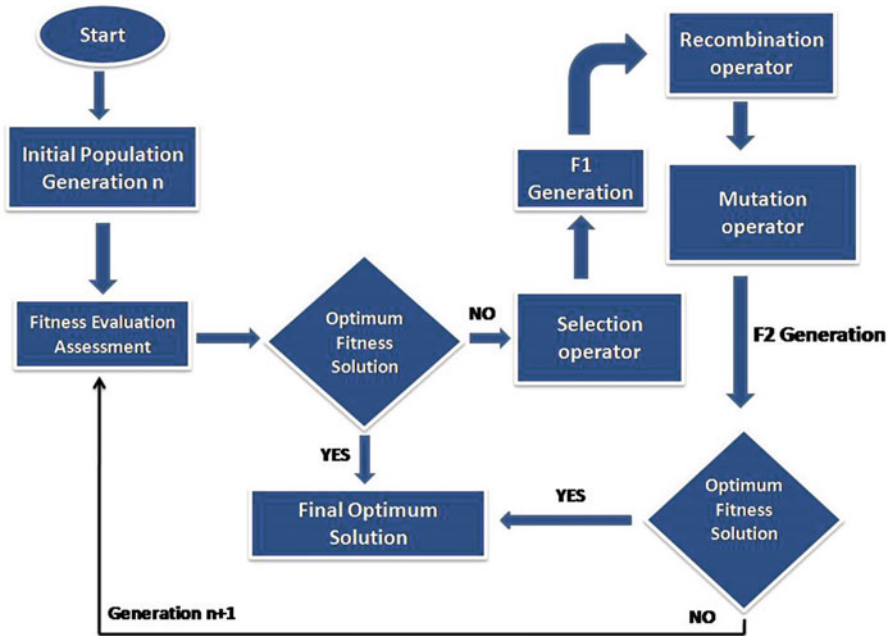


Fig. 14.1 Working of genetic algorithm

14.6 Artificial Neural Network

An artificial neural network (ANN) is a mathematical or computational model that mimics the structural and functional behavior of biological neural network. Consisting of a set of precisely designed artificial neurons, ANN works in unison to solve a specific problem. ANNs have been broadly applied with great success for system designing, modeling, optimization, and control mainly due to its capacity to learn noise filter signals and generalize information through a systematic training procedure (Singh et al. 2009). ANN appeared to be a feasible method for modeling hairy root growth and culture conditions. Neural networks are typically implemented in the estimation and multistep prediction problems even with unknown solutions but can also be used as controllers directly or as an adjuster of any process parameter for a traditional controller. In ANN artificial neurons are arranged in input, hidden, and output layers (Fig. 14.2). Almost all the computations are done in the hidden layer.

Neural networks are “trained” using a data set and then used to foretell new data points. The prior knowledge is not essential for this training as the network and system remain as a black box to the user and provide the result through its own artificial intelligence. Notable characteristics of ANNs are that they can work steadily with large amounts of data which outshine at complex pattern recognition, involve real-time operations, possess fault tolerance potential, and require no

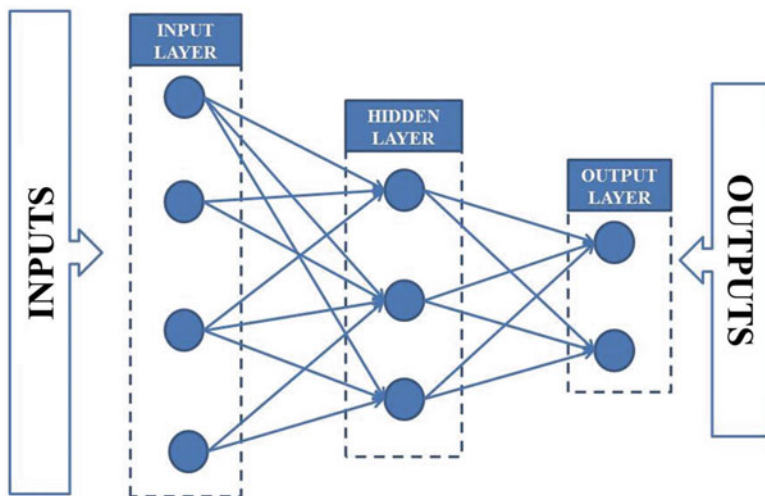


Fig. 14.2 Architecture of feed-forward artificial neural network

mechanistic description of the system. ANN is well suited for media design, as it generates a large amount of data that contains the hidden model. The “learning conditions” of neural networks are categorized into three combinations as follows:

1. Administered (associative), where the neural network is trained by providing it with input and output experimental data,
2. Self-organization in which output unit is trained to respond against clusters of pattern within the input. Different from the organized, there is no previous set of groups in which the patterns are to be classified and then the system must develop its representation of the input stimuli.
3. Support where training may consider as an ordinary form of the above two classes of learning.

ANN has been found to be well suited for hairy root growth in nutrient mist reactor as reported by Osama et al. (2013). Combination of ANN and genetic algorithm (GA) has also been found to be very effective for maximizing the native concentration and shelf life of secondary metabolites (Khan and Tripathi 2011). The ANN can perform well on nonlinear program problems and can continue working without any difficulty by their parallel nature even when an element of the neural network fails (Vaidya et al. 2003). ANNs can be implemented in a wide range of problems and do not need to be reprogrammed at every step of solution providing.

The major limitation of artificial neural networks is that they require prior data of the process. The data set used for training is very important; it decides the quality of network prediction. If the training data set is incomplete or contains wrong values, the training will be incomplete. The network will give faulty outputs.

The growth pattern in biological system is complex, nonlinear, and difficult to predict and cannot be controlled by our will. These processes are controlled by

genetic and environmental factors which are highly inconsistent (Mehrotra et al. 2008). Hairy roots are considered to be an alternate source of secondary metabolite production. In large-scale culture and production of hairy roots, the cost and the culture duration for production are very important. The conventional modeling techniques often become ineffective in monitoring and predicting the growth pattern of hairy roots. Hairy roots form heterogeneous clumps, and therefore direct monitoring of growth parameters is difficult. There is a need for indirect strategies of monitoring their growth. These may include development of models of the developmental pattern of hairy roots which can then be used for monitoring of growth. Mehrotra et al. (2008) used a feed-forward back propagation neural network to predict in vitro culture conditions for optimum biomass growth of *Glycyrrhiza glabra* plant. In other study regression and back propagation neural network was used to predict the culture parameters for maximum biomass yield for hairy root of *G. glabra* (Prakash et al. 2010). They used inoculum density, pH, and volume of growth medium per culture vessel and sucrose content of the growth medium as parameters to predict hairy root biomass. The neural network model was very efficient and was able to explain over 98% of the variations in the kinetic data. This approach was also used for modeling growth of hairy root of *Artemisia annua* in a nutrient mist reactor (Osama et al. 2013). Different architectures of ANN were compared to model reactor and several reactor parameters. All the network models were found to be efficient in modeling the nutrient mist reactor.

Artificial neural networks have also been used in combination with hidden Markov model for predicting optimum conditions for maximum biomass of hairy roots (Mehrotra et al. 2013). Five culture conditions were taken as input parameters to predict hairy root biomass. The input parameters were fed to the neural network through five HMM models. The combinatorial model proved to be efficient in predicting hairy root biomass.

14.7 Conclusion

Hairy roots cultures are fast growing and have high capacity of production of secondary metabolites. They possess several qualities due to which they are very promising candidate for large-scale production of phytochemicals. For large-scale production of hairy roots, constant monitoring of growth and phytochemical production is essential. Direct monitoring of hairy root growth in the reactor system is difficult, time taking, and labor intensive. Several indirect techniques have been proposed for monitoring hairy root growth in bioreactors. Most of these techniques require good understanding of the biological processes and effect of environmental conditions on hairy root growth. Apart from this, a good understanding of the effect of hairy root growth on their environment is also essential. Several mathematical models and machine learning-based models have been proposed for this. Machine learning techniques prove to be a promising tool for modeling complex biological process.

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