



## Review

# Scale-Up Strategies for Solid State Fermentation Systems

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*Information available on scale-up strategies for solid state fermentation systems is scarce and empirical approaches have usually been used.*

*Scale-up is further complicated by the involvement of various types of bioreactors, intense heat generation and non-homogeneity in the system. The resulting fermentation plants are, consequently, labour and energy intensive and the development of well-founded scale-up criteria, such as those for submerged fermentation processes, is vital for extensive commercialization of solid state fermentation systems.*

## INTRODUCTION

Solid state fermentation (SSF) systems, involving the growth of microorganisms on moist substrate in the absence of free water,<sup>1</sup> simulates the fermentation reactions occurring in nature.<sup>2</sup> The moist solid substrates, which are polymeric in nature and insoluble in water, act as a source of carbon, nitrogen, minerals, water and other nutrients as well as providing anchorage for the microorganisms.<sup>3</sup> These processes have been used extensively from ancient times in the Oriental, Asian and African

countries for the production of fermented foods, starter inocula, mushrooms, dough fermentations, etc.<sup>4</sup> Concentrated efforts have been made in these countries to establish automated fermentation plants in order to gain significant economic advantages over conventional submerged fermentation (SmF) processes.<sup>2,3</sup> The details of the processes and technologies are closely guarded trade secrets.

In Western and European countries, on the other hand, the SSF system was neglected from 1940 while efforts were directed towards the development of SmF.<sup>3</sup> It has been stressed that no comparison of process economics seems to have been made between SSF and SmF systems in Western and

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European countries.<sup>5</sup> This arbitrary decision may be linked to the comparatively slow growth of the fermentation industries in these countries as compared to Japan.<sup>6</sup> A critical analysis of these historical events, present day knowledge of fermentation and an appreciation of the importance of the optimum water content of the medium even in the case of bacterial fermentations indicate that decisions taken in 1940 to ignore SSF systems in these countries were probably not appropriate.<sup>7</sup>

A closer examination of SSF processes in recent years in several research centres throughout the world has led to the realization of numerous economical and practical advantages of SSF.<sup>2,3,8</sup> SSF has now been extended to bacterial and yeast cultures<sup>9,10</sup> and a wide range of products are being explored including gibberellic acid,<sup>11-13</sup> bacterial thermostable alpha-amylase,<sup>14-16</sup> cheese flavour,<sup>17</sup> ethanol,<sup>18-20</sup> tetracycline,<sup>21</sup> penicillin<sup>22</sup> and the upgrading of straw.<sup>23</sup> In spite of these advances,<sup>24,25</sup> the commercial exploitation of SSF in Western and European countries is scarce<sup>2,3,26</sup> mainly due to the dearth of well founded scale-up criteria.<sup>27</sup>

The present state of the art of scale-up strategies for SSF systems is critically evaluated in the present review. Problematic unit operations are also identified and an attempt is made to indicate possible ways for overcoming these difficulties on a large scale.

## THE SIGNIFICANCE OF SCALE-UP

Scale-up has been defined technically in a variety of ways by different workers.<sup>28-31</sup> Scale-up is not just a one-way procedure involving smaller to larger size systems but also includes the reverse, commonly referred to as scale-down.<sup>28</sup> The latter is also valuable for obtaining additional information in less-expensive ways of carrying out procedures and is characterized by simplicity and efficiency.<sup>31</sup>

Microbial fermentation process development itself is a multi-disciplinary activity involving the participation of microbiologists, biochemists, organic chemists, enzymologists and engineers with specialization in biochemical, chemical, mechanical, civil and environmental disciplines.<sup>32</sup>

The development of modern microbial fermentation processes involves many steps which have been grouped into six activities:<sup>27</sup> (a) isolation, screening and selection of the appropriate micro-organism; (b) optimization of physico-chemical and nutritional parameters as well as standard-

ization of process unit operations at a laboratory scale; (c) scale-up studies and, if necessary, the design and establishment of the pilot plant; (d) generation of engineering data and design and layout of the commercial plant; (e) construction of the plant and the solving of difficulties in plant operation during the commissioning step; (f) regular operation of the plant for the production of microbial metabolites. Scale-up is therefore the crucial link in transferring a laboratory scale process to commercial production scale.<sup>27</sup> It also provides a large quantity of the product which might be required for product evaluation and toxicological studies.

Unsuccessful scale-up results in wasted time spent on cost-intensive laboratory scale work and also forces the withdrawal of prospects thought earlier to be potentially profitable.<sup>32</sup>

## PECULIARITIES OF SCALE-UP EXERCISES

Scale-up exercises are process specific. Little will be known about the peculiarities of a particular process at the onset of the work and the process may fail at pilot plant level but work well at the production scale.<sup>33</sup> Such peculiarities might have resulted in the loss of a number of potentially valuable processes because of reverse scale effects.<sup>34</sup> In other cases, the process may not show any peculiarity in scale-up and the success can be achieved in a simple fashion.<sup>35</sup> It is generally accepted that the design of a commercial scale bioreactor cannot be accomplished solely by a purely theoretical approach.<sup>36</sup> This may explain the relative abundance of reports concerning the quantitative understanding of theoretical and chemical engineering aspects of the scale-up which has proceeded more rapidly due to the intrinsic simple nature of the physico-chemical phenomena.<sup>28</sup> The fermentation process which can be scaled-up without any difficulty is a rarity, probably because of the enormous increase in the volume or bulk of the medium, which is often 250-1000 times.<sup>32</sup>

## SCALE-UP STAGES

Scale-up involves a series of stages, the number of which have been a subject of considerable debate. In practice, the number of stages are generally determined by the type of the process and the earlier experiences of the team concerned.<sup>28,32</sup> The ultimate

deciding factor, however, is the sufficiency of data for effective scale-up exercise.

A system of four stages with the selection of one size of fermenter for each stage has been suggested for SmF processes by Bank.<sup>32</sup> This also appears to be applicable to the SSF system. In the modified form, these stages are:

(a) *Flask level*: 50–1000 g working capacity for the selection of the culture, optimization of the process and experimental variables. Data collection is facilitated in a short time and at low cost.

(b) *Laboratory fermenter level*: 5–20 kg working capacity for the selection of procedures for inoculum development, medium sterilization, aeration, agitation and downstream processing, standardization of various parameters such as oxygen transfer rate, carbon dioxide evolution rate, biomass formation, product biosynthesis profiles; studies on the effect of pH, aeration-agitation rates, continuous or intermittent nutrient feeding policies, selection of control strategies and instruments, evaluation of economics of the process and its commercial feasibility.

(c) *Pilot fermenter level*: 50–5000 kg mainly for confirmation of data obtained in laboratory fermenters, selection of best inoculum procedure, medium sterilization and downstream processing strategies. It facilitates market trials of the product, its physico-chemical characterization or toxicity testing and determination of viability of the process.

(d) *Production fermenter level*: 25–1000 tonnes for streamlining the process which ultimately leads to a financial return on the investments made so far on process development.

## PROBLEMS ASSOCIATED WITH SCALE-UP

Scale-up problems are the effects of fermentation size on process productivity<sup>28</sup> and include a number of major and minor areas.<sup>32</sup> A critical analysis of these aspects is attempted below for fermentation processes in general and SSF systems in particular.

### Variations in the biomass formed

The achievement of equivalent biomass in large fermenters and laboratory scale experiments can be assured by using the same inoculum size and identical growth medium as well as fermentation parameters due to the linear relationship between the number of generations of the cells and the logarithm of fermenter volume. Many microbial cultures are prone to variant development, however,

and this is a common phenomenon in industrial fermentations.<sup>37,38</sup> The number of variants will be higher in large fermenters due to the increased number of generations necessary to come to that stage. The variants formed in any given generation will also multiply in all the subsequent generations. This situation can have drastic effects on process productivity if the variants are non-producers or if their growth rate is faster than that of the normal cells. These will also have more pronounced effects in fermentations involving non-growth associated product formation or secondary metabolite production in the stationary phase of the growth.

These problems call for the minimum numbers of generations in the production fermenters. This cannot be achieved on economic grounds since it is essential that a high biomass is allowed to develop in production fermenters in order to achieve the targeted product formation. The presence of variants in high proportions in the biomass formed in large scale fermentations has forced the abandonment of many processes at the pilot plant level. The best solution is an extremely cautious approach in the selection of the culture during the screening programme and experimentation on the selected strain to verify variant appearance during successive serial transfers.

### Large scale inoculum development

The inoculum is generally used at a high ratio in most fermentation processes for the production of secondary metabolites, with the aim of producing the desired level of product in a short period. Similarly, most of the SSF processes involve the use of a high ratio of inoculum, but the purpose in this case is to prevent contamination during fermentation.<sup>27</sup> Consequently, inoculum production in large quantities becomes a distinct unit operation in large scale fermentations and is usually achieved by using a series of inoculum fermenters of increasing capacities. In turn, this necessitates some changes in the growth medium and the cultural parameters. For example, the test tubes, petri dishes and culture bottles, used at laboratory scale for the growth of inocula on the surface of agar media are impracticable for larger scale inoculum production and appropriate liquid cultures are used. In cases where the inoculum was grown in liquid medium on the laboratory scale work, it is possible to use the same composition of liquid medium in larger fermenters but changes such as agitation and aeration of the medium become essential. Consequently, the metabolic state of the inoculum cells or

spores, their viability and the age of the inoculum may be different from those in the inoculum grown at laboratory scale.

An additional problem of some SSF processes is the preparation of the spore inoculum which is usually preferred due to the ease of uniform mixing of spores with moist solid substrate. It becomes imperative to continue the fermentation, at each inoculum development stage, until a good crop of spores is formed. This requires 6–7 days in the case of fungal cultures. The formation of spores in the earlier inoculum development stage and their germination in the next stage can increase the frequency of mutation. The development of the inoculum at a large scale also involves an increased number of subculturings which, in some cases, leads to culture degeneration.<sup>38</sup> The policy of the minimum number of subculturings during inoculum development is therefore essential in large scale fermentations. If the culture is not able to tolerate the minimum of 4–5 subculturings required, the best approach is to discard the culture and go back to the screening stage and select another appropriate but more stable culture. Alternatively, the size of the production fermenter can be reduced or two identical units for inoculum development, working in tandem, can be employed to avoid the problem. However, these alternatives add substantially to the cost of production.

#### Medium sterilization

Medium sterilization on a large scale poses many problems such as temperature profiles, physico-chemical alterations in the medium, thermal degradation of essential nutrients, formation of toxic compounds and nutrient damage<sup>32</sup> due to the effect of scale factors. The sterilization batch time on a large scale is 3–6 h as against that of 15 min at 121°C at laboratory scale in the batch sterilization mode. The heating time to attain the desired temperature of sterilization and the post-sterilization cooling to incubation temperature are scale dependent and pose problems in batch sterilization processes. The sterilization process is also influenced by many factors such as (a) the rate of killing of the microorganisms at any time is proportional to the number of viable cells present, (b) the initial population is extremely variable in size and even its determination is time-consuming as well as being of doubtful significance, (c) the microflora present in the medium have different heat sensitivities, (d) the thermal death rate is influenced by the physico-chemical state of these

microflora, (e) the scale dependence of the logarithm of the ratio of the initial to the final population (the sterilization criterion) and (f) the necessity for the linear increase of the sterilization criterion with the logarithm of the culture volume.<sup>28</sup>

Continuous sterilization involving a high temperature–short time principle is comparatively easy to scale-up and hence adopted in many SmF processes. The probability of thermal destruction of nutritive constituents of the medium is also lower, the method is amenable to easier automatic control and offers a low turn-round time. The major problems of continuous sterilization include higher capital cost, greater risk of contamination and a decrease in medium performance with an increase in sterilization time.

Yet another problem faced in medium sterilization is related to the presence of complex organic materials in the media used at pilot and production scales. These substances and their constituents are prone to heat damage and may even interact with each other at higher temperature to form undesirable or toxic compounds.<sup>32</sup> Even the quality of the medium ingredients used in larger scale medium is different. The quality of the water in large scale media will also be different as distilled or deionized water is usually employed at laboratory scale but cannot be afforded at large scale, unless it is of critical significance or in the production of high value–low volume products. The impurities introduced in the large volume media due to these changes can cause severe problems both during growth and in downstream processing operations.

Medium sterilization in SSF processes involves the dual objectives of medium sterilization and physico-chemical changes in the solid substrate. For example, wheat bran (WB), a commonly used substrate in SSF processes,<sup>39</sup> becomes modified and more amenable for microbial growth during autoclaving. Therefore, the sterilization time used is much longer even at flask level in SSF systems as compared to SmF processes (often 60 min against 15 min at 121 °C).<sup>1,40,41</sup> It is essential that each particle of the solid substrate is heated to 121°C for 60 min in large scale sterilization. In addition, the use of continuous sterilizers at large scale pose severe difficulties due to the physical nature of the moist solid medium in SSF systems. Consequently, there is a reliance on batch sterilization. The existence of temperature profiles and the times of heating and cooling phases in batch sterilization, therefore, assume critical importance in SSF systems.

### Aeration

On a laboratory scale the requirement for aeration is met by agitating the culture flask, while in large scale fermentations forced aeration is employed. Aeration not only provides oxygen but also simultaneously removes carbon dioxide, other volatile metabolites and heat from the fermenter. The rate of aeration therefore is determined by factors such as the growth requirements of the microorganism, the production of gaseous and volatile metabolites and heat evolution. The volumetric oxygen transfer coefficient,  $K_L a$ , is universally employed as a measure of aeration efficiency in SmF processes and also has applicability in SSF systems. A reliable and efficient method for  $K_L a$  estimation in SSF systems was developed recently by Durand and coworkers.<sup>42</sup> The volumetric mass transfer coefficient for  $\text{CO}_2$  removal agrees well with the  $K_L a$  for  $\text{O}_2$  absorption, provided a correction is made for differences in productivities.<sup>28, 43</sup>

Oxygen uptake in SSF is probably directly from the gas phase and, to a lesser extent, from the liquid associated with the solids.<sup>2</sup> The confinement of gas transfer to the liquid film on the substrate surfaces was stressed by Mudgett.<sup>44</sup> Many operating parameters and medium characteristics can affect  $\text{O}_2$  transfer rates including the air pressure and flow rate, the porosity of the moist solids, the bed depth of the moist fermenting solids, perforations in the culture vessel, the moisture content of the medium, the reactor geometry and impeller rotational speed and geometry.<sup>27</sup> The agitation of the medium can also promote  $\text{O}_2$  transfer by dispersing air as small bubbles in the medium, increasing the gas hold-up time, preventing the coalescence of air bubbles and creating turbulence in the medium.  $\text{O}_2$  transfers, in general, are more easily achieved in SSF systems than in SmF media as air can easily diffuse into the inner regions of the moist solid mass.<sup>27</sup> It is well established that the  $\text{O}_2$  transfer from gaseous phase into liquid phase is dependent on the rate of oxygen dissolution in the fermentation medium, but not that from the liquid phase into the cell.<sup>45, 46</sup> However, gas diffusion problems will be encountered in static fermenters and in the cases of intraparticle diffusion. The penetrated portion of the fungal hyphae in the solid substrate particles may therefore be starved of oxygen.<sup>27</sup> Furthermore, a rapid decrease in  $\text{O}_2$  concentration across the microbial biomass in the liquid film at the solid surfaces may also be encountered as the liquid film over the substrate is relatively stagnant.

Problems are usually encountered in meeting the

aeration demand of the large fermenters. The selection of operating conditions which will support the required  $\text{O}_2$  transfer rate during scale-up presents another major problem.<sup>28, 32</sup> In general, forced aeration of the medium is advantageous as it achieves higher efficiency by combining aeration with agitation. It is necessary, however, to prevent channelling of the air flow by using suitable diffusers or other strategies such as the periodic complete change of gaseous atmosphere in the fermenter or the reversing of the direction of the air flow.<sup>47, 48</sup>

### Agitation

This is one of the most important parameters, especially in aerobic fermentations, since it ensures homogeneity with respect to temperature and gaseous environment and provides gas-liquid interfacial area for gas to liquid as well as liquid to gas transfers.<sup>28, 32</sup> Agitation also promotes surface mass and heat transfers and the uniform distribution of nutrients added incrementally during the course of fermentation.

It must be emphasized that agitation is not employed in many aerobic SSF processes such as tray fermentations which are carried out in static reactors.<sup>2, 27, 49</sup> In contrast, agitation is usually an essential part of periodically or continuously agitated SSF bioreactors.<sup>27</sup> The requirement of agitation in SSF systems is governed by the type of process, reactor design and the product concerned. A number of products such as aflatoxin, ochratoxin and enzymes are produced in greatly enhanced yields in agitated systems.<sup>50-52</sup> Agitation was also shown to be beneficial in suppressing sporulation.<sup>52-54</sup> In contrast, poorer growth of *Rhizopus oligosporus*<sup>55</sup> and decreased ethanol production by *Saccharomyces cerevisiae*<sup>56</sup> have been reported in agitated systems. Agitation is also known to have adverse effects on substrate porosity due to the compacting of the substrate particles, disruption of fungal attachment to the solids and damage to fungal mycelia due to shear forces in SSF systems.<sup>27</sup> However, no beneficial or adverse effects of agitation in the production of gibberellic acid<sup>11, 12</sup> and bacterial alpha-amylase<sup>57</sup> have been reported. Moreover, the agitation may promote or prevent aggregate formation of the fermenting mass depending on the nature of the solids.<sup>27</sup> Agitation was reported to prevent the mycelium from binding the substrate particles into a mass.<sup>51</sup>

The scale-up of agitation systems in agitated SSF processes may not pose problems because the rates

of agitation used in most of the processes are quite low.<sup>2</sup> The use of intermittent rather than continuous agitation will be more appropriate to prevent damage to the mycelia and disruption of mycelial attachment to solids.<sup>27</sup> The adequacy of periodic mixing to maintain maximum gas transfer rates and no further enhancement of oxygen transfer rate with continuous agitation have also been reported.<sup>23</sup> Similarly, a combination of agitation and heat removal for selecting the agitation rate to control the temperature of the solids at optimum level may also prove beneficial.<sup>27</sup>

#### Heat removal

A large quantity of metabolic heat is generated during the course of fermentation and its rate is directly proportional to the level of metabolic activity in the system.<sup>28,32</sup> In addition, mechanical heat is also generated due to agitation and air sparging of the culture medium,<sup>28</sup> though such heat will be of lower magnitude in static SSF system and even in agitated SSF processes due to the use of lower rates of agitation. The heat evolution rates of 3200 kcal,<sup>58</sup> 80 kcal,<sup>59</sup> 600 kcal<sup>42</sup> and 14960 Btu<sup>60</sup> per kg dry matter have been reported in SSF systems: The temperatures were higher by 17–20°C in the centre of the bed, as compared to the set value of 30°C,<sup>58,61</sup> and the temperature of 60–70°C in the innermost region of the compost as compared to that of 37 °C in the outermost region of the heap<sup>62</sup> indicates the extent of heat generation.

In laboratory scale bioreactors, heat is usually removed by placing the culture vessel in a temperature controlled room, or water bath or by passing cooling water through the baffles and/or jacket of the fermenter. This is adequate on a laboratory scale although chilled water is used in highly active fermentations. These strategies are also used in larger bioreactors with the addition of external heat exchangers. It should be emphasized that the fermenter size increases with the cube of the linear dimensions, while its surface available for cooling purposes increases with the square of the linear dimensions.<sup>28</sup> Such decreases in the availability of heat exchange surface on a large scale sets a maximum limit to fermenter size in industrial plant.

The heat removal by conduction and convection is poorer in most SSF systems due to the absence of agitation, a low rate of agitation in agitated systems and the poor thermal conductivity of the solid substrates. For these reasons heat removal has been thought to be an insurmountable problem in large

SSF bioreactors of conventional types.<sup>23</sup> Evaporative cooling has been employed in many cases<sup>28</sup> as it has been shown to be of greater efficiency than convection and conduction<sup>23</sup> and has the ability to remove up to 80% of the heat generated.<sup>63</sup> It results, however, in a large moisture loss and consequent drying of the solids.<sup>27</sup> It is, therefore, essential to combine temperature and moisture controls in SSF systems. A computer controlled, integrated temperature–moisture system and the use of a rocking fermenter have been successfully developed recently<sup>64,65</sup> and may prove useful in the scale-up of SSF processes.

#### Moisture content of the solids

Water is used in only limited amounts in SSF systems<sup>27</sup> and when available in lower or higher quantities than the optimum value affects the process productivity significantly.<sup>2</sup> The existence of an optimum moisture content of the medium, even for bacterial cultures, has been stressed recently.<sup>66</sup> Water also has profound effects on the physico-chemical properties of solids and this, in turn, affects process productivities.<sup>27</sup> It is for these reasons that the moisture content of the medium and the relative humidity levels in the fermenter have been stated to be major key factors which determine the outcome of the process.<sup>2</sup>

Various strategies such as the use of a high initial moisture content of the medium and humidification of the atmosphere in the fermenter to 90–98% have been practised to maintain the moisture content of the solids.<sup>2,67,68</sup> Evaporative cooling is the preferred method for heat removal, but it leads to uneven distribution of water, both in horizontal and vertical directions, due to evaporation of water from the system in large quantities.<sup>69</sup>

#### pH control

It is difficult to monitor and control pH in the SSF system, since pH electrodes able to measure the pH of the moist solids, in the absence of free water, are not available. The mixing of small quantities of acid or alkali with the bulk of the solids will also be highly problematical in static SSF systems. The strength of acid or alkali used for pH adjustment needs to be high to avoid drastic changes in the moisture content of the solids. An adequate procedure for measuring the pH of the sample of the fermenting solids involves the insertion of the pH electrode into the moist solids taken in the palms of both hands and noting down the constant pH reading after gently pressing the solids around the

tip of the electrode to release some absorbed water from the solids.<sup>70</sup> Alternatively a small volume of distilled water is added to the moist solids and mixed thoroughly for pH measurement by electrode,<sup>71</sup> although such values are higher by 0.1–0.2 pH when compared to the former methodology.<sup>72</sup>

The control of pH during the course of fermentation involves the inclusion of buffers in the medium and the use of urea as a nitrogen source rather than ammonium salts.<sup>27</sup> In many cases, reliance is placed on the strong buffering action of some of the solid substrates used in the SSF system.<sup>1,2</sup> The best policy for pH control in an SSF system employing evaporative cooling for heat removal involves integration with moisture control.<sup>64,65</sup> The acid or alkali at the desired concentrations is dissolved in the water used for the spraying of the fermenting solids in such a system. This technique may be used to overcome problems of scale-up.

#### Contamination control

The fermentation operations beyond autoclaving of the medium are not under aseptic conditions in many SSF processes. Consequently, contamination problems are usually experienced during scale-up of the process if due precautions are not taken. A strategy of using a high ratio of inoculum is, therefore, adopted in many processes to control the contamination.<sup>1,2</sup> A low moisture content and pH level of the medium are useful in minimizing the growth of contaminants although this may become impractical if these lower values affect the growth and metabolism of the culture. Other strategies employed to control contamination include the use of newspaper sheets or cloth to cover the substrate and the use of antiseptics such as formaldehyde<sup>73–75</sup> but these are impracticable on a large scale.

On a large scale, contamination can be effectively controlled by selecting appropriate fermenter types. For example, the closed fermenters such as rotating drums, covered pans, columns and bins can control contamination. In contrast, the open type of bioreactors such as tray fermentation are highly prone to contamination. A knowledge of the source of contamination will also be useful. The condensation of water on the ceiling in the koji room and development of contamination wherever such condensed water fell has been reported.<sup>67</sup> In this case, the placing of two rows of unperforated trays on the top of the trollies to collect such water drops, was found to be helpful. Thus, the use of closed

bioreactors, a large ratio of inoculum and good house-keeping practices may overcome contamination problems.

#### Heterogeneity

The involvement of water insoluble solid substrates, a limited quantity of water and the use of air for the supply of O<sub>2</sub> as well as evaporative heat removal lead to the presence of solid, liquid and gaseous phases in SSF systems, thereby making these highly heterogeneous. Difficulties will therefore be encountered in achieving same mass transfers and diffusions with an increase in scale. This leads to concentration gradients, a common feature of SSF systems,<sup>27</sup> and affects the process productivity to a significant extent.

#### Downstream processing

This is a highly neglected aspect of scale-up, even though its critical importance and economic implications are well understood.<sup>76,77</sup> A glance at the patent literature indicates that research in this area is often pursued in isolation by different investigators to those engaged in fermentation. This has serious implications for the process economics.<sup>78</sup> This area has been studied in depth at the CFTRI, Mysore, India.<sup>79–83</sup> It is important to note that methods of downstream processing developed for the SmF process cannot be applied directly. In SSF systems the product must be initially leached from the fermented solids. The involvement of bulky solids and the low concentration of the product in these solids may also pose many problems.

At the end of fermentation in the SSF system, the fermented mass consists of non-utilized solid substrate, microbial cells, spores (if these are formed by the culture), the product for which fermentation was carried out and a number of co-metabolites formed during the course of fermentation.<sup>71,78</sup> The product needs to be leached out from the solids with a suitable solvent. The leaching efficiency is affected by a number of factors such as reprocessing of the fermented solids, efficiency of solvents, additives to solvents, diffusivity of solute as well as solvent, retention of solvent by solids, mixing of solids and solvent, the ratio of solids to solvent, contact time, temperature and pH of the system and techniques used for separation of the leachate.<sup>78</sup> The selection of the method of leaching is also critical in terms of practicability and economics.

The efficacy of a number of leaching techniques including (a) percolation, (b) multiple-contact counter-current leaching, (c) pulsed plug flow

extraction in column, (d) hydraulic pressing, and (e) supercritical fluid extraction has been evaluated recently. The utility of the last technique is limited to low volume – high value products like gibberellic acid.<sup>82</sup> The use of percolation methods results in dilute leachate of the product (solids to leachate ratio of 1:10) and often dictates cost-intensive vacuum concentration.<sup>79</sup> It also defeats the advantage of the SSF system in giving highly concentrated product as compared to the SmF process.<sup>78</sup> On the other hand, the multiple-contact counter-current leaching technique leads to concentrated product (solid to leachate ratio of 1:1)<sup>79-81</sup> but is highly time-consuming, laborious and dictates constant attention of skilled operators.<sup>83</sup> Hydraulic pressing<sup>84</sup> requires a two stage operation for higher efficiency. The combination of multiple-contact counter-current leaching and hydraulic pressing at the end of each stage<sup>85</sup> involves bulk handling of the solids a number of times with the consequent extra cost.

Pulsed plug flow extraction in a column has emerged as a method of choice as it results in a 1:2–1:3 ratio of the solids to the leachate depending on the working parameters. In addition, it offers many advantages such as simplicity of operation, good leaching efficiency, economy, low energy input, lower capital expenditure and operating expenses and minimum labour involvement.<sup>83</sup> It overcomes the problem of moving the solids between stages and the higher viscosity of the extract which are encountered in counter-current leaching. It also facilitates the completion of the leaching process unattended between the last shift of one day and the first shift of the next day in the fermentation plant.<sup>83</sup> Moreover, the scale-up of this system may not prove problematic.<sup>78</sup>

In the case of volatile products, the methods for the recovery from the fermented solids can be of different types.<sup>78</sup> A classical example of such a SSF system is the process for the production of ethanol from a variety of non-conventional sugary and starchy substances. The policy of continuous stripping of ethanol from the solids during the course of fermentation has been adopted.<sup>63,86</sup> This can also overcome the toxic effects of high ethanol concentrations on the fermentation and become useful when the concentration of ethanol in the fermented solids is lower than the economically distillable concentration.<sup>78</sup> The method is simple and practicable but no data are available on its economics. The efficiency of recovery of ethanol from fermented solids by manual squeezing, percolation tech-

niques, hydraulic pressing and direct distillation was compared recently.<sup>18</sup> The loss of ethanol during these recovery processes was higher than the corresponding loss of ethanol from the unleached sugars or starch that would be left in the pulp if it is subjected to sugar or starch recovery operations before solid phase fermentation.<sup>18</sup>

Once the product is leached out from the solids, the leachate simulates the fermented medium obtained at the end of SmF process. The conventional methods of SmF processes for isolation, concentration, purification and formulation of the product can, therefore, be employed.<sup>78</sup> A detailed and critical review on the state of the art of leaching of the product and further downstream operations in SSF system was published recently.<sup>78</sup>

### Waste management

This is another aspect of SSF systems which has been neglected. The waste streams arising from SSF systems include spent solids, liquid effluent and exhaust gases.<sup>78</sup> The liquid effluent emanates from the washing of equipment/machinery, downstream processing steps and substrate soaking in water. The volume of liquid effluent is usually low. The exhaust gases from different sections of the plant can be connected to a central gas ducting system, discharged to the atmosphere after passage through primary and secondary filters and decontamination by UV light disinfectors.<sup>87</sup> It may become necessary to subject the exhaust gases to gravity settling, centrifugal forces, impingement and diffusion or a combination of these methods if they contain hazardous agents.<sup>87</sup>

The largest volume of waste emanating from SSF systems is the spent solids resulting from the leaching of the product from the fermented mass.<sup>78</sup> It contains the non-utilized portion of the solid substrate, microbial cells, spores (if formed), water, solvent used for leaching purposes and the unleached portion of the product and co-metabolites.<sup>78,88</sup> The BOD load will be high and could cause severe atmospheric pollution. The volume will also be large due to the lower bulk density of the solids. Negligible efforts have been applied to its management except those at ORSTOM, France. The ensiling of the spent solids, emanating from the SSF process for the production of cellulases by *Trichoderma harzianum*, was shown to yield good quality animal feed.<sup>84,88</sup>

Strategies for the disposal of spent solids also include (a) use for biogas production, composting, land-filling, ethanol production after enzymic sac-

charification and nucleic acid recovery from the spores present in the residue; (b) in the manufacture of bricks, boards and papers; (c) use as fuel, antagonistic agents to phytopathological fungi in soil; and (d) as cattle feed after sterilization and drying or after protein enrichment.<sup>1, 89-94</sup>

It must be emphasized that waste treatment processes involve high capital and constant operating expenses but do not give any return to the industry except in cases where anaerobic digestion is used to obtain biogas or the solids are ensiled or used directly as animal feed.<sup>78</sup> These expenses on waste treatment add heavily to the product cost. Hence, the selection of simpler, reliable, efficient and economic means for waste management is of critical importance. The selection of such a method can only be done during scale-up studies at pilot plant level as the waste will not be available in sufficient quantity in the preceding stages for characterization and experimentation. It is of economic interest to note that the spent residue is not generated in some SSF processes as the fermented solid itself is the final product.<sup>78</sup> The classical examples of these types of processes include those for production of fermented foods, inoculum or seed koji, enzyme koji, compost, ensiled solids and protein enriched agro-industrial products/residues.<sup>78</sup>

Various management systems such as recycle, utilization, waste exchange and reduction in the volume as well as strength of the waste are available and can be of high potential in SSF processes.<sup>95</sup> The work on recycle of stillage from SSFs for making slurries of the dry substrate and its use for leaching of sugars from the solids<sup>96</sup> or the sequential reuse of the spent solids for the production of different enzymes.<sup>97, 98</sup> deserve special mention.

#### Solid handling

The solid state of the medium in the SSF system poses severe problems of solid transfers and transportations in various unit operations, especially on a large scale. The problem is further complicated as the solid substrates used in SSF processes vary widely in size, shape, abrasiveness, shear-sensitivities and flowability.<sup>27</sup> The moisture content of the solids also influences the performance of the solid handling machinery,<sup>27</sup> and this assumes critical importance as the bulk of the solid handled in SSF processes is in moist form.

Solid handling in SSF systems can be carried out using bins or hoppers for bulk storage of the solids, feeder systems for transporting solids from the bin

for medium preparation, conveyor or elevator to move the material from one to another unit operations of the process and discharge equipment to disengage the solids from the carrier system to the desired delivery point.<sup>27</sup> These may involve pneumatic or mechanical conveying and expression as well as extrusion processes. It will also become necessary to incorporate in-line solid-liquid mixing machinery for mixing the solids with liquid during medium preparation and product leaching.<sup>27</sup> In processes involving more than one type of solid substrates in specific proportions, solid blending machinery will also be required. In addition, overall automation will be desirable, considering the need to handle large quantum of bulky solids.<sup>27</sup>

### SCALE-UP CRITERIA FOR SmF PROCESSES

#### Diversity and magnitude

The scale-up criteria available for SmF processes can be divided into two major types: (a) mathematical model dependent systematic empirical criteria and (b) mathematical model independent pragmatic empirical criteria. The existing scale-up criteria have been classified into five groups based on the approach involved.<sup>27, 30</sup> These include (a) fundamental methods, (b) semifundamental methods, (c) dimensional analysis, (d) rule of thumb and (e) trial and error techniques. Some of the major scale-up criteria of SmF processes involve the maintenance of various attributes such as (a) constant volumetric oxygen transfer rate, (b) impeller tip speed, (c) volumetric power input, (d) mixing time, (e) momentum factor, (f) shear rate, (g) kinetics, (h) agitation effects, (i) Reynolds number, (j) geometric similarity, and (k) the growth environment such as the dissolved oxygen concentration.<sup>28, 32, 99-102</sup> In addition, a scaling-down approach has been advocated<sup>31</sup> while the older approach based on trial and error efforts is still prevalent.<sup>27</sup>

#### Practicability for SmF processes

An analysis of the five groups of scale-up criteria has been performed recently<sup>27</sup> and it points out that these methods are too difficult to apply in most cases or do not always lead to satisfactory results.<sup>86</sup> Considerations based on mass, heat transfers and momentum are involved in these fundamental methods which are difficult to solve and consequently pose many problems in their practicability. The semifundamental methods overcome

these problems to a large extent due to their reliance on simplified equations and relationships. However, these are far removed from the actual situation and do not hold good at a large scale of operation in many cases. Dimensionless numbers are concerned with fluid mechanics and assure mechanical similarity.<sup>33</sup> These are more relevant to external particle mass transfers<sup>103</sup> and their utility is limited to relatively simple systems like first order homogeneous chemical reactions.<sup>33</sup> Moreover, dimensional analysis cannot be done unless enough is known about the physics of the reaction. The rule of thumb and trial and error techniques are older, time-consuming and highly cost-intensive.

#### Applicability to SSF system

For all the above reasons, near absence of fundamental as well as practical/empirical knowledge, dependence of the transport phenomena on the scale of operation especially in systems with limited water, slower inter-particle as well as intra-particle diffusion, a linear increase in time for flow transport with scale (in contrast to that for diffusional transport with the square of the scale) and high heterogeneity of the system,<sup>27,30</sup> these criteria may not prove efficient in the scale-up of SSF processes. Moreover, the applicability of dimensionless numbers can be eliminated straightaway as the particle size of the substrate does not increase in proportion to scale in SSF processes, as required for the validity of the dimensionless numbers.<sup>27</sup> The rule of thumb and trial and error methods can be used but with all their limitations and disadvantages. Another possibility is the adoption of reactor designs which have proved to be efficient and by trial and error obtaining the same productivity at small and large scale in a similar type of reactor. This approach has many limitations. For example, the reactor design which performed satisfactorily at laboratory scale may not have the same efficiency on a larger scale.

#### SCALE-UP METHODS USED IN SSF SYSTEMS

A thorough literature survey revealed that the development of scale-up criteria or its use for scaling-up of the SSF system has not been reported so far except for the recent report on the development and validity evaluation of an efficient and practicable criterion.<sup>86</sup> The SSF system is exploited commercially in many countries<sup>4</sup> and

obviously the scale-up efforts using some criteria have been used in designing industrial scale plants. Lack of information on the topic on the part of industry is understandable from the viewpoint of industrial secrecy. However, the lack of reports from universities and R&D centres indicate that the trial and error technique was probably employed.

Some of the attempts made by the authors in earlier years to develop large scale SSF processes were therefore analysed with a view to establishing the scale-up criteria involved. In addition, a typical example of the scale-up based on geometric similarity maintenance is also cited. These cases represent the scale-up trends in the development of larger and pilot scale SSF processes and systems developed by many other workers. The recently formulated scientific criterion is also described.

#### The scaling-down approach

The Central Food Technological Research Institute (CFTRI), Mysore, India, has carried out research and development on SSF systems for more than two decades and has developed a number of industrially viable technologies.<sup>1,2,25,27,104</sup> Some of these have been transferred to industry for commercial exploitation, after scaling-up.<sup>4</sup> In addition, a laboratory scale process developed by the Indian Jute Industries' Research Association, Calcutta, India, was also successfully scaled-up recently. A fermentation plant to produce 200 kg dry mouldy bran (DMB)/day was also designed and is nearing erection. This process is for the production of an enzyme complex by *Aspergillus terreus* IJIRA-6 for use by the jute industry in upgrading jute fibres and biomodification of tamarind kernel powder (used for sizing of the jute fibres).<sup>104</sup> Preliminary scale-up trials were also conducted jointly with the Central Leather Research Institute, Madras, India, on the laboratory scale process developed for the production of alkaline proteases by *Aspergillus flavus* by SSF.<sup>104</sup> This enzyme is of industrial use in dehairing hides and skins as well as the bating of leather. The laboratory scale work on all of these processes was carried out in flasks which were incubated in a slanting position to provide a larger area of substrate and a shallow depth while the koji fermenter was used in scaled-up processes.<sup>2,105</sup> A thorough analysis indicated that the criteria used were based on an environmental approach and involved experiments on a small scale under environmental conditions that can physically be realized at the production scale, i.e. the scaling-down approach.<sup>31,32,101,106-108</sup>

In the scaling-down method, a decision must be made first on which reactor will be used at an industrial scale before the process is studied on a laboratory scale. The cycle for taking the process from laboratory to industrial scale, via scaling-down involves four phases, i.e. (1) adopting the most efficient existing or contemplated industrial scale reactor design, (2) laboratory scale simulation, (3) standardization of environmental conditions and reactor operation strategy on a laboratory scale, and (4) implementation at full scale either directly or through pilot plant study. The choice of industrial scale design can be based on general or incomplete information of the microbial strain to be used, the microbial metabolite to be produced or a comparison of the reactor design in similar processes.<sup>30</sup> Such preliminary design selection also allows for an educated guess of the range of environmental conditions to be expected at laboratory and industrial scales. The environmental conditions that can be considered in the scaling-down method include oxygen transfer rate, mixing time, shear and substrate composition. This method is therefore a kind of kinetic approach but under realistic conditions.<sup>31-33, 101, 102</sup> Once the process is standardized on a laboratory scale using simulated conditions of the contemplated fermenter design for large scale fermentation, the scale-up of the process involves an increase in the bioreactor size by maintaining geometrical similarities.

The use of Erlenmeyer flasks as culture vessels for standardization of environmental and culture parameters on a laboratory scale in work at CFTRI was a simulation of the industrial scale tray fermenter or koji room. Various cultural techniques such as the use of moist solid medium in a shallow layer in the flask, the spreading of the medium on one side of the inner surface of the flask, the incubation of the flask in an inclined position and the use of an incubator with temperature as well as humidity control, were adopted<sup>1, 2, 105</sup> and duplicate the large scale tray fermentation.

#### Trial and error approach

Considerable work on protein enrichment of starch and starchy substrates at laboratory and pilot scales has been carried out jointly by the Institut Francais de Recherche Scientifique pour le Developpement en Cooperation (ORSTOM), Institut de Recherche en Chimie Appliquee (IRCHA) and Centre National de la Recherche Scientifique (CNRS) in France.<sup>109-112</sup> These attempts involved a trial and error approach in scale-up and it was stated that the

direct extrapolation from a laboratory scale column fermenter was complicated by the difficulties encountered in eliminating the calories produced during the course of growth.<sup>112</sup> Therefore, a baker's dough mixer was selected as a fermenter after extensive trials and modifications to suit the purpose.<sup>112</sup> It was considered to be the most simplified equipment possible which allowed both the homogenization of the medium and the regulation of the temperature.<sup>112</sup> The capacity selected was 55 litres and it was further increased to 500 litres, maintaining geometric similarity. Subsequently, the plant of capacity of 1 t/day was developed in collaboration with Societe Pour l'Equipement des Industries (SPEICHIM), France.

The trial and error method involves continued attempts with rectification of minus points at each stage until success is achieved. Takamine<sup>75</sup> has described the scaling-up process for fungal alpha-amylase production. This paper gives a good insight into the trial and error method and also shows the benefits of ingenuity.

#### Maintenance of geometric similarity

One of the classical examples involving this criterion for scale-up is the process for cultivation of microorganisms in an air-solid fluidized bed.<sup>113</sup> An experimental bench scale fermenter of 16 litres capacity, equipped for control of temperature, humidity and aeration rate, was used to cultivate *Aspergillus sojae* or *Aspergillus oryzae* on wheat bran powder for cell mass and enzyme production. It performed much better than the conventional SSF fermenters and offered the control of operating conditions as easily as in SmF processes because of sufficient mixing.<sup>113</sup> After preliminary experiments on the effects of the increase of tower diameter, layer height of fermented substrate packed in the bed in a fluidized state, temperature distribution and metabolic calorific values on process productivity, the fermenter was scaled-up to 3300 litres capacity by maintenance of geometric similarity.

This scale-up technique works on the basis of using the same physical proportions during scale-up to pilot and industrial plants. In the case of SmF processes, the same ratio of tank dimensions and internals are maintained. Moreover, the impellers are sized and placed in a similar way, although the impeller-to-tank diameter can be varied to some extent.<sup>32, 101</sup> This method was commonly used in the case of SmF processes prior to 1948 but was subsequently replaced with other efficient principles. It is a simple method but suffers from severe

limitations and no guarantee can be given of the similar performance at large scales.<sup>101</sup>

#### Maintenance of constant heat and water balances

This is the outcome of the combined efforts by researchers from different laboratories solely for the development of simple and efficient scale-up criteria for SSF systems.<sup>86</sup> The criterion was derived by analysing all the factors which affect SSF, their relative role and the conditions that would assure maximum possible productivity. The criterion involves the maintenance of heat and water balances in the system at all stages of scale-up and has emerged as a winning candidate. It was evaluated for ethanol production by *Schwanniomyces castellii* in column fermenters using sugar-cane bagasse as an inert solid to absorb liquid nutrient medium. The selection of this process for validity evaluation of the criterion was guided by various scientific, technological and industrial reasons. The validity evaluation studies indicated similar trends in four different sizes of column fermenters with respect to total carbohydrates, starch, maltose, glucose, pH, biomass formation, biosynthesis of ethanol, and concentration of CO<sub>2</sub> and O<sub>2</sub> in the exhaust air.<sup>86</sup> The fermenter sizes employed involved 6-410 gravimetric scale factors from 10 g moist substrate size and increases in the diameter as well as height of the columns.<sup>86</sup> The data indicate a high potential of this simple scale-up criterion which was not used earlier for scale-up of any fermentation process.

The scale-up criterion involving maintenance of equal heat and water balances is based on the use of forced aeration of the solids by humidified air for evaporative cooling to maintain the temperature of the solids. The humidification of the air and the recirculation of the cooled exhaust gases also ensure control of the moisture contents of the solids. The rate of aeration is sufficient to meet the demand of the culture for oxygen.<sup>86</sup> A computer controlled integrated program and control systems are available to control efficiently the temperature and the moisture of the solids.<sup>64,65</sup> Thus, the criterion is practicable and may prove to be the key for extensive commercialization of SSF processes.

#### NEED FOR DEVELOPING MORE SCALE-UP STRATEGIES

Among the scale-up strategies used so far in SSF systems, only two, which advocate the maintenance of the same heat and water balances and the

scaling-down approach, have a scientific base. These are simple, reliable and practicable. Different types of bioreactors are used in SSF systems and it will be advantageous if scale-up strategies for each type of reactor are available. It is concluded that a need exists to formulate defined criteria for all round commercial exploitation of SSF systems.

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