# Metabolic Products of Mixed Culture Fermentation

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Siddhartha Pandey, Nitin Kumar Singh, Tara Chand Yadav, Ankur Kumar Bansal, Arti Thanki, Manish Yadav, and Jayato Nayak

**Abstract**

Mixed culture fermentation provides a great avenue for the production of various metabolites of commercial use. It has clear economical and process advantages over pure culture fermentations. However, some limitations still exist as the consortium of bacteria drives the process kinetics. To overcome such limitations, the bio-processes as well as syntrophic relationship of the microorganisms must be taken into account to control the process meticulously. In this chapter, we have focused mainly on mixed culture fermentation, complexities associated to it, control and regulation of the process and several parameters which play a vital role in stable operation of the mixed culture fermentation process. This chapter

S. Pandey
Department of Civil Engineering, Chalapathi Institute of Technology, Guntur, Andhra Pradesh, India

N. K. Singh (✉)
Department of Environmental Science and Engineering, Marwadi University Rajkot, Rajkot, Gujarat, India
e-mail: nitinkumar.singh@marwadieducation.edu.in

T. C. Yadav
Department of Biotechnology, Indian Institute of Technology, Roorkee, India

A. K. Bansal
Department of Civil Engineering, Moradabad Institute of Technology, Moradabad, Uttar Pradesh, India

A. Thanki
Department of Environmental Science and Engineering, Marwadi University, Rajkot, Gujarat, India

M. Yadav
Central Mine Planning and Design Institute, Bhubaneswar, Odisha, India

J. Nayak
Department of Chemical Engineering, VSB Engineering College, Karur, Tamilnadu, India



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aimed at understanding the functionality and importance of solid-state fermentation, along with submerged fermentation.

**Keywords**

Mixed culture · Metabolic products: fermentation · Metabolism regulating parameters · Solid-state fermentation

## 5.1 Introduction

Production of fermentation products through anaerobic conversion of organic substances is one of the potential biotechnological processes. In general, fermentation is a biological catabolism process and controlled through the substrate-mediated redox reactions. More specifically, the generation of adenosine triphosphate (ATP) takes place in the absence of commonly used external electron acceptor such as $O_2$, $NO_3^-$, or $CO_2$ (El-Mansi et al. 2006). Although, substrate works as primary sink for the electrons, but excess electrons may be utilized for the production for elemental hydrogen through reducing protons. In such processes, the conversion of potential energy to cellular energy is facilitated by oxidation reactions, especially in the form of ATP. This mechanism is propagated in literature as substrate-level phosphorylation. Literature review revealed that requisite energy can also be leveraged by fermentation cells via alternate ways of electron transfer, i.e. cations and protons-mediated process, and other electron transport chains (Konings et al. 1994).

To date, accurate prediction of fermentation products is quite strenuous, especially for the mixed culture-based systems. Industrial fermentation processes are typically performed using pure cultures and aimed at the production of high-value products. For bulk chemical production, the pure culture process seems less attractive due to their elevated financial requirements, particularly associated with the controlling mechanism of the culture performance and its strictly sterile working conditions in order to prevent contaminations. Important equipment investments are necessary for these processes at industrial scale. In addition to this, pure culture fermentations require generally the use of pure and therefore more expensive substrates. The risk of contamination of the culture furthermore remains since an unstable pure microbial culture is used. Mixed cultures comprise of a consortium of stable and mixed microbial species, typically found in nature. The use of less pure substrates (even wastes or by-products) is possible with the subsequent cost implications. Mixed culture fermentations (MCF) did not find wide application at industrial scale because they present still important limitations. Further, great variation in qualitative and quantitative characteristics of MCF is observed in some studies, which suggest that maintaining the optimum balance between mixed culture microorganism required a thorough understanding of behaviour of associated microorganisms.

## 5.2 Production of Metabolic Products from Mixed Culture Fermentations (MCF)

From the industrial processes, large amounts of residues are produced as waste. The further utilization of these wastes by the biotechnological industry is quite limited. The major obstacle identified as presence of diversified organic compounds in these waste residues. Production of energy carriers (Claassen et al. 1999) or other valuable products by mixed culture fermentations would bring utility to those useless wastes or by-products and also enable interesting downstream integrations. MCF is a potentially interesting technology for validation of these streams and generation of specific products. Products that can potentially be obtained by MCF include mixtures of volatile fatty acids, alcohols, lactate that may serve as building blocks in other processes. Several interesting applications exist for MCF processes.

(a) Production of biodegradable polymers such as 3-hydroxyalkanoicacids (PHAs) and poly-3-hydroxybutyricacid (PHB) has been extensively investigated (Lee 1996). PHAs can effectively be produced by mixed cultures of bacteria by imposing a strong selection pressure on the mixed culture (Reis et al. 2003).
(b) Biological hydrogen production by MCF has a large research interest (Benemann 1996) due to its potential application as energy carrier. The yield of hydrogen depends on composition of formed fermentation products and their stoichiometric quantities.
(c) Solvent fermentations, for the production of alcohols and acetone, butanol or propanol by using clostridial cultures, have been of increasing interest in the past few decades (Dürre 1998). These processes have been proven effective in the development of sustainable additives to gasoline. To date the extent to which mixed culture can be applied to the production of specific solvents remains largely unclear.
(d) Furthermore, the carbohydrate fermentation is a crucial step in valorization of solid waste streams as well as anaerobic digestion of applied wastewater. In anaerobic digestion processes of wastewater, the initial fermentation of carbohydrates, known as acidogenesis, leads to a wide variety of products that are subsequently methanized by using other microbial consortia. The interest of carbohydrate fermentations in the framework of all these applications motivates for modelling these processes from the perspective of control of the product formation (Singh et al. 2016).

If anaerobic bioconversion process is carried out in multiple steps, various intermediate by-products can be separated out through favourable pathways of individual substrate conversion. Such specifically designed bioreactors warrant an enhanced biodegradation of organics along with the increasing yield, selectivity and improved qualities of product. With this vision, two-stage processes are marked by obvious advantages over single-stage anaerobic systems, as two-stage processes can guarantee an enhanced production of gaseous products, i.e. methane and hydrogen, and other desired products/outputs such as bioplastics, bio-flocculants, bio-polymers, bio-pesticides, biosurfactants and energy. Therefore, by offering an

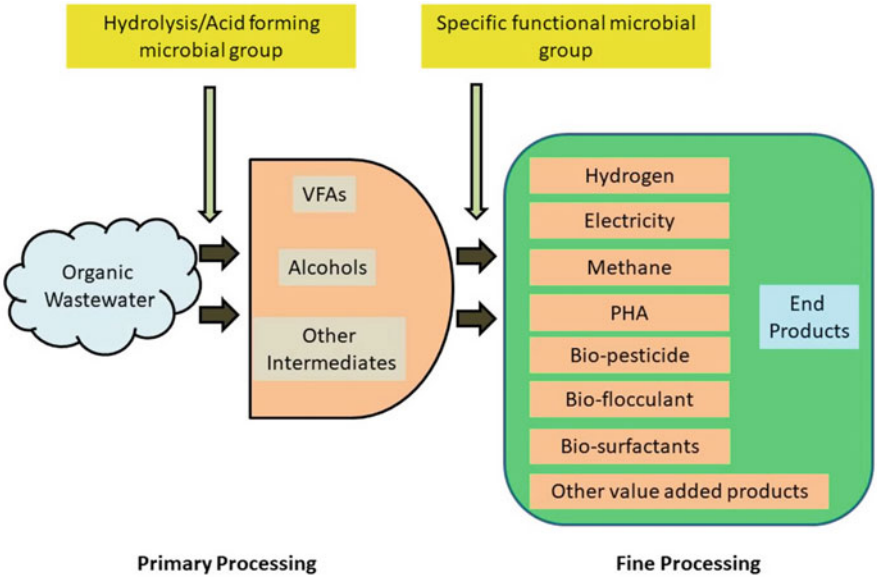


**Fig. 5.1** Schematic of fermentation process showing possible biochemical end products recovered from wastewater

appealing avenue, it can provide a promising platform, probably a practically viable and economically feasible, for future applications. However, there are also various challenges which remain as a hurdle in such path-breaking developments (wen-wei Li et al. 2011). Figure 5.1 represents a schematic depicting the biochemical products oriented possible pathways and energy recovery from an anaerobically treating two-phase wastewater system.

## 5.3 Complexities Reported in Mixed and Pure Culture-Based Microbial Fermentation Processes

Generally, microbial communities of anaerobic systems do not utilize the external electron acceptors. Instead, these microbial populations are expected to generate the larger part of their ATP through a well-known mechanism designated as "substrate-level phosphorylation". Further, in the absence of substrate-level reduction, utilization of excess electrons can be facilitated by the substrate reduction mechanism which lead to the production of light weight compounds such as short chain fatty acids, sugar alcohols, and some other alcohols. Some researchers also reported that hydrogen gas can also be produced through such electron transfer mechanism. Thus, fermentation processes are mediated through coupled substrate-level oxidation–reduction mechanisms. More specifically, oxidation reactions (or substrate-level phosphorylation) provide the required catabolic energy, whereas reduction reactions are supposed to be favouring the sinking of electrons. Moreover, excess electrons

can be sunk through hydrogen, however; thermodynamically/energetically seems to be unfavourable, especially at high partial pressure of hydrogen (Rodríguez et al. 2006).

One of the considerable examples of pure culture-based processes is production of bioethanol from starch by using a model organism *Saccharomyces cerevisiae*. This process is rapid in nature and can be inoculated with well-acclimatized cultures. This pure culture-based system is able to generate desired quality and quantity of products, even in the presence of inhibitory/competing species. Thereby, high yield of products can also be expected in the absence of expensive sterilization equipment (Lin and Tanaka 2006).

On the other side, in pharmaceutical production, stringent guidelines of production such as complete elimination of contaminating/secondary microorganisms, are imposed by Food and Drug administration of United states and some medical agencies of European countries. With respect to uncontrolled and high-rate fermentation of sugars through mixed cultures, microbial contamination can be expected via undesirable side reactions of various microorganisms. This behaviour can largely be attributed to the insufficient knowledge about the control of mixed culture-based systems (Ciani et al. 2010). More specifically, lack of knowledge about the factors affecting product spectrum is mainly responsible for failure of mixed culture system. In spite of these challenges, mixed culture-based systems are reported to have few secondary products such as propionate and lactate (Eng et al. 1986; Horiuchi et al. 2002). To date, biotechnological products such as acetate, butyrate, and ethanol have been reported to be produced from glucose, in controlled mixed culture fermentation processes (Temudo et al. 2007). Besides, published research studies lead to the development of fermentation models, which can be accurately used for the prediction of specifications of products at set pH range and with low substrate concentration (Rodríguez et al. 2006).

## 5.4 Factors Affecting Regulation of Metabolism in Mixed Culture-Based Systems

To date, various factors have been reported to affect the functioning and control of mixed culture system. Some of the important factors include pH, temperature, hydraulic loading, organic loading and type of substrate (Himmi et al. 2000; Voolapalli and Stuckey 2001; Batstone et al. 2002). The variation in aforementioned parameters can directly affect the product spectrum as well as microbial diversity. Each of this factor has the tendency to impose the effect on cellular metabolism or ecology of the systems. Results of such interaction have been reported to be producing wide ranges of products.

### 5.4.1 Role of pH and Its Effect

The pH of media plays a vital role in qualitative and quantitative characteristics of mixed culture fermentation system. In particular, the rate of dissociation of microbially produced organic acids is reported to be dependent on pH levels of media. Further research investigations revealed that the dissociation rate can directly affect the production of ATP, required for the cell maintenance (Rodríguez et al. 2006). With respect to undissociated organic acids, favoured by low pH conditions, diffusion of these forms of acids into the cell takes place passively. This led to the requirement of increased rate of active transport (ATP-consuming) to facilitate the removal of acid products. Such conditions of energy requirements may direct the cells to adopt a secondary ATP generation mechanism, i.e. shifting from acetate pathway to butyrate pathway with lower ATP yield at low pH conditions. The stoichiometric conditions of butyrate pathway have also shown lessen impact on pH per mole of glucose. In this way, the active energy transport requirements are reduced. This shift in metabolic pathways (acetate to butyrate) for mixed culture systems has also been reported in scientific literature by various researchers (Temudo et al. 2007). Some authors also reported that at low pH conditions, mixed culture can also maintain ATP generation through acetate pathways by increasing the pH neutral ethanol (Ren et al. 1997). Regardless of this, it is still a matter of research that what type of conditions causes mixed cultures to produce butyrate even at low pH in some cases, and ethanol in some another studies. This metabolic behaviour change may be linked with the type of used substrate. Temudo et al. (2007) observed such behaviour with glucose as sole carbon source, while possible results were reported by the Ren et al. (1997) for the combination of sucrose and molasses. Additionally, one of the exceptional results was also published by Horiuchi et al. (2002), in which higher yield of propionate was reported at a pH of 8, with fermentation of glucose through mixed cultures. However, these results have not been replicated till now.

### 5.4.2 Effect of HRT and OLR

Hydraulic retention time (HRT) and organic loading rate (HRT) are well-known operational parameters of biological systems. The change in these parameters is also found to be associated with metabolic activities of pure and mixed culture systems. Both OLR and HRT ensure the availability of substrate in continuously operated biological systems. So far, OLR effect, which is considered as an important primary variable and represents a combined effect of applied substrate concentration and HRT, has not been investigated much in fermentation processes. However, it is considered as principal lumped variable which determines the maximum cellular flux or available feed for microbial communities. Therefore, many scientists and engineers have argued about the effect of OLR in mixed culture systems. We, as an author, also into the perusal of OLR effect, as a controlling factor in fermentation metabolic processes. Till now, researchers have reported the effect of OLR on mixed

culture, mainly in the anaerobic digestion process and defining the limits of methanogenic activities. Eng et al. (1986) reported that increased OLR of sucrose in an aerobic digestion process can led to the high yield of lactate and propionate. This subsequently changes the yield of methane and $H_2$, probably due to inhibited dissociation of these products. The research studies of Voolapalli and Stuckey (2001), based on shock loads of glucose and sucrose, revealed that VFA production can increase in such case. However, a difference in acid products in not discussed in this study. Further examinations were also conducted at increased OLR with constant substrate concentration. Agler et al. (2012) reported that production rate of fermentation products such as butyrate can be increased by varying the HRT with constant OLR conditions (maintained by varied substrate concentrations). These findings revealed that both the HRT and OLR can affect the metabolic pathways of mixed culture systems. Further, it should also be investigated whether there will be a difference between varying OLR with constant HRT and varying HRT with constant OLR conditions. Overall, it has been observed that very limited published work is available in this direction and thus there is ample opportunity to conduct research on this topic (Pandey and Sarkar 2017).

### 5.4.3 Role of Substrate Type

Like pH and loading rates, choice of substrate has also an impact on metabolic activities of fermentation processes. The experimental investigations on the effect of substrate type in fermentation processes are well-documented by various researchers (Ren et al. 1997; Temudo et al. 2007; Lu et al. 2013). The results of these studies revealed that there may be a difference in the yield of ethanol and butyrate, especially at low pH conditions. In particular, the degree of reduction of substrate depends on its type, which consequently led to the production of reduced EMCs with differing quantities. As shown in Fig. 5.2, each substrate has a different tendency to enter the fermentation metabolism. Hence, each substrate offers a different pathway for the production of metabolic products and their quantities. Furthermore, inhibitory effect can be observed during some particular pathways for some substrates. One of such examples, reported by Cameron et al. 1998, include inhibition of sugar for the 1,3-propanediol pathway. The research studies, based on pure culture, have shown extensively that product spectrum can vary significantly according to the choice/type of substrate. One of such study was conducted by Lewis and Yang (1992), in which varying product spectrum was achieved by *P. acidipropionicion* glucose, lactose and lactate. Further examination of this study revealed that in case of lactate as fermentation substrate, highest and lowest production was observed for propionate and acetate, and succinate, respectively. Yu et al. (2007) also demonstrated that glucose and lactose have different abilities of affecting fermentation gene expression for *Clostridium acetobutylicum*. Detailed analysis of this research study revealed that genes, responsible for lactose fermentation, can be expressed in the sole presence of lactose. On the contrary, when a mixture of glucose and lactose is provided, genes responsible for lactose fermentation were not expressed until a sufficient amount of

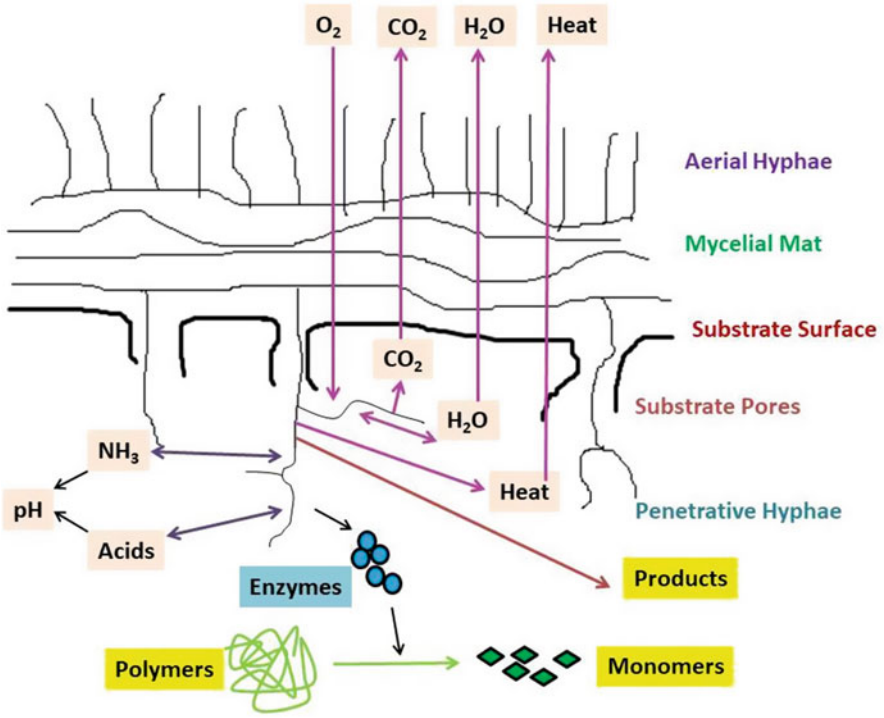


**Fig. 5.2** Microscale processes occurred during solid-state fermentation

glucose had been consumed. It is clear that a change in product spectrum is dependent upon substrate types as well as its composition. Further in-depth examinations revealed that choice of substrate mainly affects the NADH:NAD+ ratio of *C. acetobutylicum* and *E. faecalis*, respectively (Girbal and Soucaille 1994; Snoep et al. 1991). However, these results were indecisive, as change in ratio of substrates was a direct or indirect relation with relative redox state of the substrate. Such observations may be attributed to the fact that catabolic pathways are microorganisms as well as substrate-specific. One of such cases include bioethanol industry, where fermentation of xylose was observed to be negligible in the presence of strains of *S. cerevisiae* (Jeffries 2006; Ha et al. 2011). Such behaviour of a particular species may be linked with the role of selective pressure maintained in the mixed culture system. Recent published works also revealed that substrate plays an important role in the growth of microbial communities of anaerobic digesters (Zhang et al. 2014).

### 5.4.4 Effect of Temperature

Like other operational parameters of biochemical systems, temperature is one of the inevitable parameters which has profound effect on biodiversity of systems as well as product spectrum. In particular, the temperature conditions provide selective pressure for psychrophiles, mesophiles, thermophiles at $<20$ °C, 30–40 °C, and $>50$ °C, respectively (Pervin et al. 2013). With reference to chemical reaction engineering principles, temperature has a direct relation with the activation energy of biochemical reactions and thus alters the thermodynamic equilibrium. The experimental investigations of studies, based on ethanol production using *S. cerevisiae*, revealed that substrate consumption rate and product yield increased with increase in temperature. On the contrary, the yield of volatile by-products (e.g. acetaldehyde, isobutanol, VFAs) was found to be inversely proportional to the degree of temperature (Bakoyianis et al. 1997; Galanakis et al. 2012). The studies performed using mixed culture reported that fermentation rate increases with the increase in the temperature of system. However, as compared to temperature, the individual product responses, i.e. VFAs and $H_2$, only were found to be strongly dependent on pH of the system (Infantes et al. 2011). Detailed examination of such type of studies revealed that high temperature mainly favours the oxidative reactions or production of hydrogen over reductive reactions (Batstone et al. 2002). Another study reported that thermodynamic effect of temperature was found to be associated with cellular membrane permeability. The same was evidenced by Bischof et al. (1995) in their research study, performed by using fluorescent dye at different temperatures using two types of animal cells. Infantes et al. (2011) also reported that membrane permeability increases with temperature, which significantly reduces the substrate consumption at elevated temperature and low pH conditions. Unfortunately, no published literature could be found mentioning the relationship between temperature and membrane permeability of fermenting microorganisms.

### 5.4.5 Control with Electro-Fermentation

The application of requisite negative potential, through a particular electrode, to fermenting cultures has been proven effective in desired product spectrum. Such approach was found to be effective in achieving the high yield of reduced products. Some of examples include increased yield of propionate and ethanol by *Propionibacterium freudenreichii* and *Clostridium thermocellum* and *S. cerevisiae*, respectively (Emde and Schink 1990). In this regard, two mechanisms of electron uptake by cells are suggested. These are known as direct and indirect transfer of electro-fermentation. In direct electron transfer mechanism, interaction of redox active components (EMCs and/or nanowires) which are present in outer membrane of cell is reported in literature too. This mechanism is particularly hypothesized for anode-driven systems (Bond and Lovley 2003; Reguera et al. 2005; Gorby et al. 2006). Further, cathode-driven systems are also reported to be governed by this mechanism (Rabaey and Rozendal 2010; Lovley et al. 2011). Unfortunately, authors

could not find any published work highlighting the applicability of this mechanism in fermentation processes.

On the contrary, indirect electron transfer refers to the transport of electrons between microbial cultures and the electrode. This transport is facilitated by redox active shuttling molecules through the diffusion process in culturing medium. The shuttling molecules generally works as electron mediator in this process. Some of these examples include secondary metabolites such as phenazine, flavins, hydrogen produced at cathode surface, and added synthetic molecules (Marsili et al. 2008). Some of the reported synthetic electron mediators for cathodic systems are anthraquinone 2,6-disulfonic acid, methyl viologen, and neutral red (Emde and Schink 1990). Noting that control of mixed culture system is one of the major hurdles in successful implementation, synthetic mediators may play a crucial role in the regulation of extracellular electron transfer in fermentation processes. This may be attributed to their selective ability for desired value of reduction potential. As, each carrier may have a distinct stoichiometry, the process control can be governed by the energy levels at which the electrons enter into the cells. Dennis et al. (2013) reported that cathodes may be used for desired product spectrum in mixed culture fermentation, whereas, mediators with negative reduction potential were found to be associated with higher yield of products in pure culture systems (Emde and Schink 1990). These advanced strategies, i.e. use of mediators of increased negative reduction potential, can explore dimensions in making more thermodynamically favourable reductive metabolic pathways.

## 5.5 Two-Phase Anaerobic Reactors for Mixed Culture Fermentation (MCF)

In many cases, when the hydrolysis and fermentation processes are rate-limiting steps, anaerobic wastewater treatment process carried out in two stages is considered to be effective. The two-stage anaerobic reactor is comprising of two reactors in series where the first stage includes processes of hydrolysis/acidogenesis and another for specific product generation. However, an insignificant acetogenesis and methanogenesis also take in the first stage. By separation of stages, the control over optimum environmental conditions becomes easy and this enables to solve the problems associated with the microbial activities and their growth kinetics by facilitating an optimized environmental condition for each group of bacteria in each reactor (Pandey and Sarkar 2019a). Separation of stages of microbial processes increases the overall stability of the anaerobic treatment which is difficult to achieve in traditional delicately balanced single-stage anaerobic reactors. So far, various researchers have been reported the successful application of two-stage anaerobic reactors, which entails the benefits of treating waste under thermophilic and mesophilic conditions of temperature mesophilic. However, very low solid containing wastes are preferable for using two-phase digestion processes.

Till now, many modified versions of two-stage anaerobic digestion processes have been proposed, in which acidogenic and methanogenic processes are separated

and the first stage was optimized for thee agricultural and food process wastes, municipal food waste (Kim et al. 2004), demonstration of promising utilization of feedstocks such as glucose and production of hydrogen. As the less $NO_x$ is produced in this process, it gets cleanly combusted than the methane gas. As per the reports of Cooney et al. 2007, methane produced using two-stage anaerobic digestion has more stability and is significantly more effective than the methane produced in single-stage anaerobic reactor.

Two-phase anaerobic wastewater treatment can be operated in thermophilic or mesophilic conditions of temperature. High rate of biogas production adversely affects sludge settle ability as it carries over biomass excessively and finally it gets washed out. This phenomenon could be overcome by using either granulation of sludge, encapsulation of biomass, and/or biofilm formation. Nevertheless, operation under high temperatures negatively affects the granulation of sludge as the degree of mineralization of sludge is comparatively high in thermophilic reactors. Thus, a mesophilic range of temperature increases the process stability as compared to thermophilic conditions (Pandey and Sarkar 2019b). The production of extracellular polymeric substance is adversely affected by mineralization which ultimately restricts firm and dense sludge granulation. As a result of this, insignificant granulation or even degranulation takes place when mesophilic sludge inoculum is utilized as seed. Thus, with systems achieving higher concentrations of biomass, low efficiencies of treatment are expected. Van Lier (1996) reported that suspended as well as fixed-film growth reactors have encountered such problem at thermophilic temperature conditions. Further limiting the application of thermophilic anaerobic reactors industrially due to excessive biomass washout, environmental sensitivity, waste heat availability, feed characteristics variation resulting in unsatisfactory treatment performance, and degraded effluent quality.

For an enhanced propionic acid metabolism, microbial proximity, configuration of the reactor, nutrient supplementation, and characteristics of the substrate are equally important factors to be addressed (Speece et al. 2006). The rapid consumption of hydrogen by homoacetogens or methanogens is important for the oxidation for anaerobic degradation intermediates. Bioreactors such as up-flow anaerobic sludge blanket reactor, expanded/static granular sludge bed reactor, biofilm reactors (anaerobic filter or fluidized bed reactor), and membrane bioreactor provide an excellent opportunity for the proximate growth of a diversified microbial community. Therefore, to maintain the low hydrogen partial pressure, high-rate anaerobic systems are considered to be extremely efficient.

## 5.6 Solid-State Fermentation

Fermentation process in which solid matrix is used as substrate with very less water is known as solid-state fermentation (SSF). The substrate should however essentially be moist enough for the growth and metabolism of microbes. In this process, the solid matrix can itself be a source of nutrients. In many cases the substrate does not

provide nutrients, but it is impregnated with proper nutrients to support the proper growth of the microorganisms (Hoelzle et al. 2014).

The scheme of micro-processes involved in SSF has been shown in Fig. 5.2. The solid substrate acts as a support to the mycelial mat where the fungal hyphae develop. The development of hyphae protrudes from the mycelial mat into two directions. Hyphae protruding towards gaseous space are called aerial hyphae, and hyphae protruding towards moist pores of the substrate are called penetrative hyphae. When the moisture level is normal, the penetrative hyphae and mycelial mat remain in contact with water, whereas the aerial hyphae remain in contact with air. As shown in Fig. 5.2, substrate surface and inner pores support the major metabolic process. Some of the metabolic processes can also occur in the exposed region of mycelium, e.g. aerial hyphae, and it also facilitates the transport of the substances from the penetrative hyphae to aerial hyphae. Mycelium also produces hydrolytic enzymes which diffuse through the solid matrix and further helps the hydrolysis process by catalysing the macromolecules degradation into simple monomers. These simple monomers are easy to assimilate further by the fungus. In this process oxygen is consumed, and many fermentation products are produced along with $O_2$, $CO_2$, $H_2O$, and heat. The transport of gases and moisture takes place due to the development of gradient between the substrate subsurface and aerial hyphae. The major problem with the SSF process is development of heat near the substrate surface. The removal of heat either takes place via conduction or evaporation, loss of heat. The evaporation process also acts to balance the water in the system. The water is consumed during hydrolysis reaction and the same is produced back during the respiration process. The decrease in pH is also an important factor which arises from the exchange of ammonia and production of organic acids near the substrate surface. During the metabolic process, many products of interest are also released on the surface of solid matrix which needs further separation and downstream processing. Along with all these processes, many other factors, reactions, and physicochemical changes can also influence the process of SSF.

The primary aim which makes SSF a promising technology is that it allows microorganism to remain very close to the substrate as the nutrients remain in highest concentration at the solid matrix surface. It allows microbes to get nutrition easily and also favours them in a way that it resembles to the natural habitat of the microbes which adds on to their ease in growth. Biotechnology industries have now started looking forward to this technology as this provides a great avenue for the production of value-added by-products like biofuels, industrial chemical, food, secondary metabolites, and pharmaceutical products. SSF gives advantage over submerged fermentation which is the most attractive feature of it. The major applications of SSF process lie in the field of bio-pulping, bioleaching, bioremediation, etc. SSF process can directly utilize wastes generated from agro-industries as substrate which is an additional advantage as these residues can be used efficiently without contributing to environmental pollution.

The SSF technology is very much suitable for the production of secondary metabolites as solid support provides a great support for the growth of mycelium of the microorganisms. On the other hand, submerged fermentation has to be highly

**Table 5.1** Examples of secondary metabolites produced by specific solid-state fermentation

| Product | Microorganism | Substrate | References |
|---|---|---|---|
| Cephalosporin | *Streptomyces clavuligerus*, *Cephalosporin aermonium* | Barley | Jermini and Demain (1989) |
| Aflatoxin | *Aspergillus niger* | Cassava | Barrios-González et al. (1990) |
| Ergot alkaloids | *Claviceps fusitormis*, *C. pupea* | Bagasse | Hernández et al. (1993) |
| Mycotoxin corn | *Aspergillus flavus* | Wheat, oats | Hesseltine (1972) |
| Penicillin | *Penicillium chrysogenum* | Bagasse | Barrios-Gonzalez et al. (1988) |
| Tetracyclines | *Aspergillus* | Sweet potato | Yang and Ling (1989) |
| Zearalenone | *Fusarium moniliforme* | Corn | Hesseltine (1972) |

viscous in order to favourably support the production of secondary metabolites which further interferes with the transfer of oxygen. Furthermore, the secretion of secondary metabolites and filaments attached to the microbes may further increase the viscosity of the media which causes detrimental effect on the overall process. SSF technology allows for better circulation of oxygen and has many other benefits over submerged fermentation (SmF) process (Singh et al. 2018). Some secondary metabolites produced by SSF process have been tabulated in Table 5.1.

### 5.6.1 Biological Features

The advantage of SSF processes lies in providing a natural habitat to the microorganisms. Microbes like *Ascomycetes*, *Basidiomycetes*, and *Deuteromycetes* are found in nature on wet substrates in terrestrial habitats. SSF is widely as well as preferably used for the production of mould cheese in food industries, for which conidiospores of *Penicillium roquefortii* or *P. camemberti* are required. SSF is also used for the production of protection agents for plants which use *Coniothyrium minitans*. SSF also facilitates the option of growing microorganisms in mixed culture which is advantageous for many processes. A broad spectrum of enzymes is secreted by fungal consortia during their growth, which causes synergetic increase in the activity of individual enzyme. Thus, SSF has a potential to increase the productivity of the target metabolite (Singhania et al. 2009). In addition to this, SSF favours to the possibility of controlling the water activity as a selection parameter during the co-cultivation of fungi as different fungi have different water demands. In food industry, SSF finds its widespread and important application as these processes demand use of mixed cultures which is essential for a particular flavour of the food produced. For example, during the fermentation process of bamboo sprouts, a wide array of metabolites is produced along with 29 different volatile substances which are responsible for its aromatic properties.

### 5.6.2 Ecological Features

SSF process is devoid of free aqueous phase which boons this process with a minimum utilization of water which accounts for the very low amount of wastewater produced during the process. SSF is a highly environmentally friendly process as it produces low waste as compared to SmF process. Additionally, as the SSF process is carried out using less water, the chances of contamination of the system by bacteria and yeast are minimized. This further reduces the demand of energy-intensive sterilization processes. SSF process can directly use the wastes from agricultural processes as a source of nutrition, and plant residues are also frequently used for the production of enzymes and organic acids as a carbon source, which makes the SSF process environmentally friendly and sustainable.

### 5.6.3 Engineering Features

SSF process has yet to find its widespread application in western countries as it lacks standardization, low amenability, and limited reproducibility of the results. Due to lack of understanding of the processes involved and bacterial activity in consortium, the scale-up of SSF process is hard to achieve (Pandey et al. 2016). The control of the process at industrial scale of operation is a difficult task as gradients like humidity, temperature and substrate concentration cause adverse effects on the overall process. Parameters like oxygen level, temperature, and moisture content are interrelated and contribute to the difficulty in regulation of these parameters. Aerobic reactions also occur during the growth of the microorganisms, which liberates heat and ultimately causes rise in temperature. Excess heat is detrimental for the enzymatic activities involved in the SSF process as enzymes produced during fermentation process get denatured easily and become inactive at the end of the process.

### 5.6.4 Economic Viewpoint

As described previously, the SSF technology has many environmental and biological benefits which from economic point of view are very much advantageous to use. The SSF technology has been estimated to be 100 times more efficient than the SmF for the production of crude enzyme cellulose. There are multiple reasons for SSF to be a highly efficient technology. The substrate required to run SSF process is very cheap, and in fact organic wastes can be used very efficiently for the economic benefits. As the utilization of water by this process is very low, thus SSF process does not get contaminated easily and hence it saves the cost for the sterilization, energy equipment, and instruments. For the same reason, it also saves cost in subsequent downstream processing. Due to the characteristics of solid substrates for containing very high concentrations of enzymes, the cost for concentrating the enzymes during product purification is also saved.

## 5.7 Conclusions

A wide range of fermentation metabolic products of economical and commercial value can be produced from organics present in waste. Mixed culture fermentation requires little control over pure culture fermentation; however, better understanding of the various physicochemical and biological parameters plays a key role in successful operation of the process. Parameters like pH, organic loading, hydraulic loading, substrate type and temperature play key role in the regulation of a metabolic process, and these become even more critical in case of mixed culture fermentation as bacteria involved may have different response to the varying physicochemical parameters. However, solid-state fermentation has a clear advantage as it provides the bacteria a natural habitat and better control over the process. Scale-up of SSF process is still a challenge for researchers as it causes detrimental effect on product formation. Thus, for a controlled and effective operation, a better understanding is essential.

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