Synergism of microorganisms and enzymes in solid-state fermentation of animal feed. A review

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ABSTRACT. Fermentation and enzymolysis are commonly used biological treatment methods to treat forage, especially unconventional one. Both these treatments can improve the nutritional value of the forage, reduce the content of anti-nutritional factors and ameliorate the digestibility of feed. Microbial-enzymatic synergism treatment constitutes an organic combination of fermentation and enzymolysis, which can strengthen the processing of forage and achieve a superior feeding effect. In this review, the objectives of microbial-enzymatic synergism treatment are summarized, including decomposing macromolecular nutrients, decreasing anti-nutritional factors and increasing specific target products. The substrates, microbes and enzymes used in microbe-enzyme synergy are also summarized. Furthermore, the similarities and differences between one-step and two-step technological processes of synergy are presented and the current evaluation system of microbial-enzymatic synergism treatment is reviewed. Existing problems and future development directions of microbial-enzymatic synergism treatment are also discussed.

Introduction

Solid-state fermentation (SSF) has been researched and applied in the food industry for a long time and constitutes a feed material processing technique. An important objective of SSF is the production of enzymes, organic acids and other metabolites of economic importance. It was indicated that the utilization value of some substrates was increased by post-fermentation by probiotics (Chu et al., 2017; Chuang et al., 2019). Recently, SSF has been employed to enhance nutrient bioavailability, inhibit gut pathogenic bacteria and reduce anti-nutritional factors content in plant protein sources, resulting in improved nutrient digestibility, thereby improving performance and gut health of domestic animals (Olukomaiya et al., 2019). In the fermentation process, the utilization efficiency of microorganisms on the macromolecular material in feed can be improved by using enzymatic co-fermentation. For example, the monosaccharide produced by cellulase hydrolysis of crude fibre can provide energy for probiotics to synthesize microbe protein (Ghosh et al., 2019). Simultaneously, co-fermentation can also overcome the challenge of less enzyme production by microbial fermentation alone, and improve feed quality. This advantage is of great significance for the development and application of feed sources (Bartkiene et al., 2018; Wang et al., 2019). So, the main purpose of this review is to summarize the SSF method in which enzyme and microbe are used simultaneously in feed processing and to evaluate their simultaneous effect on feed SSF. Moreover, the basis for the follow-up of the rational use of enzyme in feed SSF is provided.

Purpose of microbe and enzyme cooperation

At present, the cooperative treatment of feed and feed raw materials by microbe and enzyme is a necessary supplemental measure for microbially fermented feed or enzyme-treated feed. Its primary purpose is to fully degrade the substrate by combining enzyme and microbe to develop new feed resources, improve the nutritional value of substrate feed, reduce anti-nutritional factors in substrate feed and enhance the flavour and palatability of feed (Bartkiene et al., 2018). For instance, the soyabean meal is a high-quality plant protein feed, but it contains a variety of anti-nutritional factors limiting its nutritional value. However, the impact of antinutritional factors can be decreased and the nutritional value can be improved by adding fibrolytic enzymes and lactic acid bacteria to the fermentation process (Cheng et al., 2019). By such treatments, the protein of soyabean meal can be degraded into numerous kinds of small peptides by fermentation, as well as the content of angiotensin-converting enzyme can be increased to ensure animal health (Wang et al., 2017). After the co-fermentation and decomposition by *Lactobacillus plantarum*, *Bacillus subtilis* and *Saccharomyces cerevisiae*, soyabean meal can be widely used in piglet feeding without affecting the daily gain of piglets, but augmenting their immune ability and promoting the development of small intestinal epithelial cells (Zhu et al., 2017). Indeed, the effect of soyabean meal co-fermented by protease and probiotics was shown to be superior to single fermentation or enzymatic process in broilers (Cheng et al., 2019). It has also been reported that, from the perspective of nutrition, the proportion of nutrients, and especially amino acids proportion of some inexpensive and single protein feed materials, becomes balanced after the co-fermentation by *B. subtilis*, protease and pectinases (Goodarzi Boroojeni et al., 2017; 2018).

Another main purpose of microbe and enzyme synergism is to increase the yield of specific products and obtain a specific value of substrates (Dai et al., 2017; Vong et al., 2018). The most common way to achieve this is to use protein raw materials as a substrate to produce various functional peptides and amino acids through the co-decomposition of macromolecular proteins by microbe and enzymes (Mukherjee et al., 2016; Dai et al., 2017; Reddy et al., 2017; Lee et al., 2019). The content of total phenols and flavonoids in soyabean meal and its by-products can be also significantly increased by

the synergistic treatment of microbe and enzyme (Dai et al., 2017). As these substances have strong antioxidant properties, they can be used as a component in functional food for both humans and animals (Yang et al., 2019). In a study involving a gramineous crop, sweet sorghum was used as a substrate, and a large amount of butyric acid was obtained during its fermentation by *Clostridium thermophilus* after pretreatment with trypsin and organic acid (Wang et al., 2015a). In oil crops, the extraction of camphor seed oil was increased by synergizing of *Bacillus amyloliquefaciens* and proteinase after physical and chemical mutagenesis to stimulate strains and protease activity (Zeng et al., 2017). In the field of bioenergy, to obtain bioethanol the castor cake was fermented with *S. cerevisiae* in the synergism with the cellulase (CMCase) of *Pseudomonas* (Abada et al., 2019). Similarly, maize cob was fermented by *Escherichia coli* to obtain bioethanol after being hydrolyzed by hydrolase and saccharified by a saccharifying enzyme (Pedraza et al., 2016).

Substrates, microorganisms strains and enzymes

The substrates for fermentation characterized by microbe-enzyme synergism are primarily oil cake feedstuff, fibrous feedstuff, grain by-products and animal by-products (Wang et al., 2015b; Atuhaire et al., 2016; Chuang et al., 2019) (Table 1).

The strains for microbial-enzymatic synergism in SSF are mainly animal probiotics, such as lactic acid microbes, *Bacillus*, yeasts and some moulds (Jazi et al., 2017; Yasar and Tosun, 2018).

The enzymes used in the synergism of microbe and enzyme in feed processing were primarily hydrolases, which specifically decompose substrates in the feed (Wang et al., 2017; Su et al., 2018; Cheng et al., 2019). They are also requisite enzymes for animals to digest these substrates. Therefore, according to the summary of literature, the enzyme in synergism can play an important role in the substrate of synergism, but also be an important source of digestive enzyme for animals.

All kinds of microbe may be used in the microbe-enzyme synergism of oil-cake feedstuff to enhance flavour, reduce anti-nutritional factors and improve digestibility (Shi et al., 2016; Odinot et al., 2017; Ohara et al., 2018); however, the most of the collaborative enzymes are proteases which are used to decompose macromolecular proteins in oil-cake

Table 1. Substrates, microorganisms and enzymes used in the synergistic microbial-enzymatic solid-state fermentation

Substrates	Microorganisms	Enzymes
Soyabean (*Glycine max*) meal	*Bacillus subtilis* (Feng et al., 2007; Dai et al., 2017; Wang et al., 2017; Cheng et al., 2019; Salim et al., 2019), *Lactobacillus plantarum* (Mukherjee et al., 2016; Wang et al., 2016; Zhu et al., 2017), *Lactobacillus paracasei* (Su et al., 2018), *Pediococcus acidilactici* (Wiseman et al., 2017), *Saccharomyces cerevisiae* (Zhu et al., 2017), *Clostridium butyricum* (Su et al., 2018), *Bacillus amyloliquefaciens* (Yang et al., 2019), *Aspergillus niger* (Ohara et al., 2018)	protease (Wang et al., 2017; Su et al., 2018; Cheng et al., 2019; Yang et al., 2019), acid protease, neutral protease, alkaline protease, cellulase (Salim et al., 2019; Ohara et al., 2018), α-amylase (Salim et al., 2019), xylanase (Wiseman et al., 2017), β-glucosidase (Ohara et al., 2018)
Rapeseed (*Brassica napus* L.) meal	*Saccharomyces cerevisiae* (Chiang et al., 2010), *Lactobacillus fermentum, Enterococcus faecium, Bacillus subtilis* (Chiang et al., 2010), *Aspergillus oryzae* (Dossou et al., 2019), *Aspergillus niger* (Shi et al., 2016; Odinot et al., 2017; Tie et al., 2020)	endoglucanase, acid protease (Shi et al., 2016; Tie et al., 2020), feruloyl esterase (Odinot et al., 2017), lignocellulosic hydrolyzing enzymes, phytase (Shi et al., 2016)
Cottonseed (*Gossypium arboreum* L.) meal	*Bacillus subtilis, Aspergillus oryzae, Aspergillus niger* (Jazi et al., 2017; Tang et al., 2018), *Candida utilis* (Xiong et al., 2016)	alcalase, flavourzyme (Tang et al., 2018), amylase, pectinase, cellulase, xylanase (Xiong et al., 2016; Olukomaiya et al., 2019)
Wheat (*Triticum aestivum* L.) bran	*Saccharomyces cerevisiae, Aspergillus oryzae* (Chuang et al., 2019), *Trichoderma pseudokoningii* (Chu et al., 2017)	phytase (Chuang et al., 2019), cellulase and xylanase (Chu et al., 2017)
Palm kernel (*Trachycarpus fortunei*) cake	*Lactobacillus plantarum* (Lee et al., 2019), *Paenibacillus curdlanolyticus* (Alshelmani et al., 2014)	hemicellulase (xylanase, mannanase), cellulase, proteolytic (endoprotease) (Lee et al., 2019; Olukomaiya et al., 2019)
Peas (*Pisum sativum* L.)	*Bacillus subtilis, Bacillus licheniformis* (Goodarzi Boroojeni et al., 2017; 2018)	α-glucosidase, protease, pectinase (Goodarzi Boroojeni et al., 2017; 2018)
Barley (*Hordeum vulgare* L.)	*Lactobacillus plantarum, Rhizopus oryzae* (Wang et al., 2019), lacto-acid bacteria (Yasar and Tosun, 2018)	glucoamylase (Wang et al., 2019), cellulose (Yasar and Tosun, 2018)
Grain (*Olyza sativa* L.) by-product	*Pediococcus acidilactici* (Bartkiene et al., 2018)	xylanase, cellulase, β-glucanase (Bartkiene et al., 2018)
Potatoes (*Solanum tuberosum* L.)	*Lactobacillus plantarum* (Du et al., 2018)	cellulose (Du et al., 2018)
Maize (*Zea mays* L.) stalk	*Chaetomium*, white-rot fungi, *Lactobacillus plantarum* (Atuhaire et al., 2016), *Bacillus licheniformis* (Alokika and Singh, 2019)	cellulase, xylanase (Alokika and Singh, 2019)
Maize (*Zea mays* L.) cob	*Bacillus subtilis* (Jia et al., 2017), *Bacillus licheniformis, Lactobacillus plantarum, Saccharomyces cerevisiae* (Alokika and Singh, 2019)	xylanase (Alokika and Singh, 2019), cellulase, hydrolysis enzyme (Jia et al., 2017)
Alfalfa (*Medicago sativa* L.)	*Lactobacillus plantarum, Pediococcus pentosaceus* (Chen et al., 2019), yeast, lacto-acid bacteria (Ding et al., 2013), *Lactobacillus buchneri* (Kung et al., 2003)	cellulase, hemicellulose (Chen et al., 2019), viscozyme (Schmidt et al., 2001), plant enzyme (Ding et al., 2013), β-glucanase, α-amylase, xylanase, and galactomannase (Kung et al., 2003)
Blood meal	*Bacillus subtilis* (Wang et al., 2015b), *Aspergillus niger, Aspergillus oryzae* (Zheng et al., 2014)	hydrolase (Wang et al., 2015b)
Feather meal	*Aspergillus niger* (de Oliveira et al., 2019), *Bacillus* (Bernardo et al., 2019)	protease (Bernardo et al., 2019), lipase, phytase, keratinase (de Oliveira et al., 2019)

feedstuff and improve digestibility or increase the content of specific functional peptides (Wang et al., 2017; Su et al., 2018; Cheng et al., 2019). This is essentially the same process that occurs in the treatment of animal by-products (Bernardo et al., 2019).

In the collaborative treatment of fibre feedstuff and grain by-products, the used microbes are largely *Bacillus*, yeast and mould, which are employed to disturb fibre structure and release nutrients. The used enzymes are mostly non-starch polysaccharides enzymes, such as cellulase and hemicellulase. They can decompose macromolecular polysaccharides into monosaccharide, which are used by animals or provide energy for collaborative microorganisms (Xiong et al., 2016; Olukomaiya et al., 2019).

The technology of microbial-enzymatic synergism

The synergism of microbe and enzyme in feed processing is often realized in the SSF because the amount of treated substrate is normally large (Moniruzzaman et al., 2018). At present, most of the technologies of synergism of microbe and enzyme in feed processing comprise one-step fermentation, i.e. simultaneous fermentation of substrate by bacteria in enzyme presence (Ohara et al., 2018; Salim et al., 2019), as shown in Figure 1A. It is worth noting that great changes have occurred in the process parameters when using different strains and enzymes for collaborative treatment. These parameters include the fermentation mode (anaerobic, aerobic) for the synergism of microbe and enzyme, and the determination of certain physical parameters, such as temperature, water content, process duration, inoculation amount, enzyme addition amount and initial pH of synergistic treatment (de Brito et al., 2017). Indeed, the appropriate parameters of fermentation markedly improve the treatment effect. In the determination of optimal process parameters, the research on the background of the synergistic relationship between microbe and enzyme is crucial. The parameters should fulfil the best conditions for the synergistic effect of microbe and enzymes. For example, Wang et al. (2015a) stated that to obtain a high yield of butyric acid from synergistically fermented sweet sorghum with pretreatment of *Clostridium thermobutyricum* and trypsin the temperature 50 °C should be used.

It has also been previously reported that substrates can be fermented by a second fermentation or in a two-step collaborative process (Coban and Demirci, 2014). The general process is to create the optimal reaction conditions for one kind of microbe and enzymes in the initial step and create the optimal reaction conditions for another kind of microbe and enzymes in the second step. Such a process requires changing conditions at various stages to decompose the substrate (Figure 1B). Through these two steps, two sets of process parameters are used to complete the matrix treatment more thoroughly or obtain more sufficient target products (Coban and Demirci, 2014; Tie et al., 2020). Tie et al. (2020), however, reported that the two-step method of the synergistic effect of microbe and enzyme on rapeseed meal differs from that described above. Specifically, they found that the rapeseed meal was fermented in suitable conditions for the fermentation by *Aspergillus niger*, and then the rapeseed meal was treated by the endogenous enzyme produced by *A. niger* in the second fermentation process. In addition, the temperature of the enzymolysis process was much higher than that of fermentation (45 °C and 30 °C, respectively), and the content of glucosinolates and phytic acid in the rapeseed meal was greatly decreased and the content of small peptide was greatly increased by the two-step method (Tie et al., 2020).

These results demonstrate that the two-step process could be more effective for the degradation of anti-nutritional factors and the improvement of the nutritional value of feedstuff in comparison to the use of the SSF method alone (Tie et al., 2020).
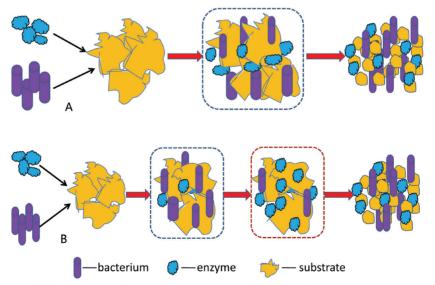

Figure 1. Process diagram of different solid-state fermentation methods with microbiological-enzymatical synergism: one-step fermentation (A) with stable parameters through the whole process and two-step fermentation (B) with changing process parameters. The dotted lines in different colours represent different process parameters.

For specific fermentation products, the two-step collaborative treatment process can be used in obtaining more fermented products with higher purity. However, the fermentation process of the two-step collaborative treatment is complex and the parameters vary markedly, which imposes high requirements for production equipment and processing technology (Odinot et al., 2017). The one-way fermentation process, on the other hand, is relatively simple and more pragmatic in feed preparation. As long as the living background and reaction conditions for microbe and enzyme are totally elucidated, better fermentation effects will be achieved after conditions are fully attained as possible.

Evaluation of the synergistic effect of microbe and enzyme

At present, the evaluation methods of the synergistic effect of microbe and enzyme in feed can be approximately divided into synergistic treatment effect evaluation and application effect evaluation. The evaluation of synergistic treatment can be summarized as the evaluation and determination of fermentation indexes, as well as the evaluation of specific products of synergistic enzymes (Wang et al., 2019). This comprises the evaluation of the fermentation index of the cooperative treatment with lactic acid bacteria as the main strain, including the pH of the substrate, the number of viable strains in the substrate and the total titrable acidity (Bartkiene et al., 2018; Su et al., 2018). The evaluation of the fermentation indexes of synergetic treatment with *Bacillus* as the main fermentation strain includes, e.g., the amino acid composition of the substrate, and the content of acid-soluble protein or specific functional peptide. (Hmidet et al., 2019; Orts et al., 2019). The evaluation of synergetic treatment indexes with yeast as the main strain includes among others yeast quantity and oligosaccharide content (Teng et al., 2017). In addition, in the indexes of fermentation and enzymolysis, conventional nutrients are commonly used to evaluate the treatment effect, such as crude protein, ether extract, crude fibre, starch and sugar. Also, in addition to these mentioned conventional nutritional indicators, some new substances in barley after the synergistic effect of microbe and enzyme were analyzed by using second derivative spectrum analysis (Yasar and Tosun, 2018).

The application evaluation is an application of the substrate treated by synergism of microbe and enzyme in animal feeding experiments. Observing and determining growth performance indexes (e.g., daily gain, feed intake, feed return), production performance indexes (e.g., egg production, milk production, slaughter rate, net meat rate), and physiological and biochemical indexes (e.g., immune factor, blood index, intestinal flora, intestinal structure) (Xiong et al., 2016; Wiseman et al., 2017). The synergistic effect of microbe and enzyme was evaluated by measuring the digestion and utilization of feed before and after synergistic treatment, such as apparent digestibility and poultry apparent metabolisable energy (Ahmed et al., 2014). For ruminants, the evaluation indicators are more related to the rumen, including the composition of rumen volatile fatty acids and rumen gas production (Mohd Azlan et al., 2018; Chen et al., 2019). It is worth noting that, in recent years, both the feed of microbe and enzyme synergism and biological fermentation feed, have achieved excellent application effects in aquaculture (Dossou et al., 2019).

Conclusions and prospects

The synergism between microbe and enzyme is a complex process of microbial life activity and biochemical reaction. Through synergy, many original substances are degraded (i.e. anti-nutritional factors) and new substances are produced (i.e. monosaccharides). In comparison to independent processes of fermentation and enzymolysis, such a synergistic approach can be more effective. In the field of animal production, it can mean the production of feed of better quality and with higher nutritional value, and so improved performance and health of animals. However, such feed processing technology is neither simple nor easy, because, in the process of synergy, different microorganisms and different types of enzymes may be involved. In the future, there is still a lot of research to be conducted on the microbial-enzymatic synergy. As for microorganisms and enzymes, the future research should focus on their living background, so that the expected synergistic effect is achieved, and on the selection of the most suitable microorganisms and enzymes for synergism. A complete set of process parameters should be formulated for different substrates, microorganisms and enzymes combinations, including among others strain, source and dosage. Moreover, simple and rapid methods for the evaluation of the efficacy of the microbe-enzyme synergism should be proposed in the experiments on animals as the final step.

Conflict of interest

The authors declare that there is no conflict of interest.

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