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Review

Co-culture: A great promising method in single cell protein production

Asmamaw Tesfaw and Fassil Assefa*

Department of Molecular, Microbial and Cellular Biology, College of Natural Sciences, Addis Ababa University, P. O. Box 1176, Addis Ababa, Ethiopia.

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The term single cell protein (SCP) refers to the dried microbial cells or total protein extracted from pure microbial culture (algae, bacteria, filamentous fungi and yeasts) which serves as food or/and feed supplements. Different substrate and fermentation optimizations are being carried out to maximize SCP production. However, little attention was given to coculturing. SCPs are produced better using coculture than monoculture. This paper reviews the positive roles of coculture in SCP production. First, it results in better saccharification of substrates and efficient carbon source utilization. Second, filling substrate utilization gap is another contribution of coculture. Third, it upgrades biomass and enrich SCP with nutrients than monoculture. Fourth, it reduces fermentation time and production cost by reducing cost of substrate treatment.

Key words: Single cell protein, biomass, coculture, monoculture, mixed culture.

INTRODUCTION

A major problem facing the world, in particular the developing nations, is the explosive rate of population growth. The Economic and Social Affairs of the United Nations (ESAUN) in 2011 reported that the number of humans in the world now totals over 7 billion; it is increasing by approximately 77 million annually (ESAUN, 2011). Although fertility levels continue to decline (ESAUN, 2011), it could exceed 9 billion by 2050 and 10 billion by 2100 (Leridon, 2008; ESAUN, 2011). Conventional agriculture may well be unable to supply sufficient food (in particular protein) and to satisfy such demands (Smith, 2000). The Food and Agriculture Organization (FAO) already predicts a widening of the protein gap between developed and developing countries. The problem becomes more severe due to arid or changing climate; infertile lands hamper productive agriculture (Smith, 2000).

Hence, alternative protein source improved in quality is a must to deal with such problems. Single cell protein is one of the alternatives that cannot be affected by climate change. SCP has a high content of protein containing all the essential amino acids. Microorganisms are an excellent source of SCP because of their rapid growth rate, their ability to use very inexpensive raw materials as carbon sources, and the uniquely high efficiency, expressed as grams of protein produced per kilogram of raw material, with which they transform these carbon sources to protein (Glazer and Nikaido, 2007). It was produced using a monoculture starting from World War I for long period for

*Corresponding author. E-mail: astesfa@yahoo.com. Tel: +251- 911956817. Fax:+251 116812065.

animal feed. However, recently co-cultures were employed since it has so many positive effects as compared to monocultures.

In nature, many species of microorganisms coexist by interacting with each other. Many species of microorganisms are most effective only when they are present in association with other groups of organisms. Mixed culture fermentations are those in which the inoculum consists of two or more organisms and are widely used in many processes including the production of antibiotics, enzymes, several types of fermented food, composting, dairy fermentation, bioconversion of apple distillery, single cell protein production, and domestic wastewater sludge (Alam et al., 2003; Gutierrez-Correa and Tengerdy, 1998). As compared to monocultures, mixed cultures of fungi may lead to better substrate utilization, increased productivity, increased adaptability to changing conditions and increased resistance to contamination by unwanted microbes (Alam et al., 2003). Nutritional limitations may be overcome in synergetic interactions between compatible partners (Gutierrez-Correa et al., 1999). The synergistic reaction occurs as a result of sequential, cooperative action between the enzymes components where the product of one enzyme reaction becomes the substrate for another (Ryu and Mandels, 1980). This paper reviews the positive roles of co-cultures in SCP production.

SUBSTRATE SACCHARIFICATION ENHANCEMENT

The presence of lignin in lignocellulosic biomass leads to a protective barrier which prevents enzymes from being accessible to cellulose and hemicellulose for hydrolysis. Lignin can be efficiently degraded by mixed microbes particularly white rot fungi. Bio pre-treatment is normally conducted at low temperatures and low pressures without using expensive equipment, chemical agents, reactors and additional energy for lignin removal and biomass structure destruction. Therefore, it is a green, safe, and inexpensive method (Tian et al., 2012). Co-cultures were more effective in substrate saccharification, which ranged between 85-88% as compared to the 62 - 67% saccharification shown by the monocultures (Eyini et al., 2002).

Cellulose and hemicellulose degradation

Cellulose is the most abundant organic compound on earth. Therefore, utilizing it as a substrate for SCP production is really what is advised. Since cellulose is a large polymer, it cannot be absorbed directly by microbes and hence it should be solublized by enzymes. The efficiency of cellulose hydrolysis requires the synergistic action of a cellulase system containing endocellulase, which cleaves internal glycosidic bonds; exoglucanase, which cuts the cellulose chain from either the reducing or non-reducing end; and β-glucosidase, which hydrolyses cellubiose to produce glucose (Bhat and Bhat, 1997; Duff and Murray, 1996).

The β-glucosidase contained in most *Trichoderma* derived cellulase was insufficient to hydrolyze the cellubiose to glucose (Stockton et al., 1991; Tangnu et al., 1981; Wen et al., 2005) and this is due to the deficiency of β-glucosidase. This leads to the accumulation of cellobiose (Stockton et al., 1991). Cellobiose is a strong inhibitor of endo and exoglucanases and the accumulation of cellobiose significantly slows down the overall hydrolysis process (Howell and Stuck, 1975). Several approaches have been attempted to overcome this deficiency. For example, a temperature and pH cycling strategy was applied to the culture *Trichoderma reesei* RUT-C30 to increase β-glucosidase production (Tangnu et al., 1981). In another study, the mutant *Trichoderma* E12 was grown on microcrystalline cellulose with peanut cake being used as a nitrogen source to obtain a high C/N ratio. As a result, a well balanced ratio of β-glucosidase activity to filter paper activity was observed (Cochet, 1991). Additionally, the problem was tried to solve by adding β-glucosidase to cellulases from either external sources (Khan et al., 1985), or by using co-culture systems (Duenas et al., 1995; Duff et al., 1987; Gutierrez-Correa and Tengerdy, 1997; Juhasz et al., 2003). Here, *Trichoderma* could be co-cultured with the fungi *Aspergillus,* which is a good producer of β-glucosidase (Duenas et al., 1995; Duff et al., 1987; Gutierrez-Correa and Tengerdy, 1997). The mixed culture of *T. reesei* and *A. phoenicis* could produce cellulase containing a high level of β-glucosidase from dairy manure; the hydrolysis efficiency (in terms of glucose produced) by the mixed enzymes was higher than those by commercial enzyme and enzyme from the single culture *T. reesei* (Wen et al., 2005). Similarly, when *Aspergillus niger* was used in combination with *T. reesei* M , production of both cellulase and xylanases on water hyacinth substrate was enhanced considerably (Deshpande et al., 2008). They postulated that the synergistic effect may be attributed to the β-glucosidase production by *A. niger* which could eliminate the inhibitory effect of cellobiose.

The cellulases obtained from simultaneous compatible mixed cultures of *Aspergillus niger* MSK-7 and *Trichoderma viride* MSK-10 have 59-66% more cotton saccharifying activity as compared to their pure cultures and other combinations (Ikram-ul-Haq et al., 2005). Likewise, *A. niger* produces a strong activity of β-glucosidase (Rashid et al., 1997), which causes deglycosylation of substrates and produces gentiobiose, a strong inducer of cellulases (Suto and Tomita, 2001). It was realized that co-culturing *A. niger* and *T. reesei* produces cellulases in substantial amounts (Juhasz et al., 2003). The synergistic interaction of these two strains in submerged fermentation led to a more efficient cellulose degradation than using either *T. reesei* or *A. niger* mono-cultures, owing to the complementary interactions of *T. reesei* cellulases and the β-glucosidases

of *A. niger* strain for complete cellulose hydrolysis (Ahamad and Vermette, 2008). The enzyme activity and fungal biomass accumulated over time suggesting that there is a strong relationship between cellulase synthesis and the amount of total biomass formation (Ahamad and Vermette, 2008). That is why the dry biomass production in mixed culture of *T. reesei* and *A. niger* (21.4 g L^{-1}) as significantly increased by 91% as compared to that of mono-cultures, 11.2 g L^{-1} (Ahamad and Vermette, 2008).

On another study, it was found that highest β-glucosidase, α-cellobiohydrolase, β-galactosidase, and laccase activities were found for *A. oryzae* in combination with other fungi, in particular with *Phanerochaete chrysosporium* and highest β-xylosidase activity was obtained when *A. niger* was co-cultivated with *P. chrysosporium* on wheat bran (Hu et al., 2011). Therefore, the protein content of *A. niger* and *Magnaporthe grisea* co-culture was doubled as compared to respective monocultures on wheat bran at 25°C (Hu et al. 2011). Likewise, van Wyk (1999) investigated the effect of cellulases mixture from *Penicillium funiculosum* and *T. reesei* on used paper products (foolscap paper, filter paper, newspaper and office paper) degradation; he found that the mixture is an effective way to increase the degree of saccharification in equal cellulase combinations (1:1). For example, this combination caused a 32% increase of glucose production relative to the amount formed by *P. funiculosum* cellulase and 72% more than the amount produced by cellulase from *T. reesei* while acting on foolscap paper. With filter paper as substrate the increase was 34% higher than obtained with *P. funiculosum* cellulase and 82% more than that which resulted from the action of *T. reesei* cellulase. With newspaper, a 59% increase to *P. funiculosum* action and 65% higher value to *T. reesei* cellulase action was observed while this combination caused 310% more glucose formation from office paper than observed with *P. funiculosum* and 50% more than that produced by *T. reesei* cellulase.

What is discussed till now is enzymatic scarification of cellulose using two strains co-culture. However, Kato et al. (2004) investigated a four-strains-mixed-culture consisting of *Clostridium straminisolvens* CSK1, *Pseudoxanthomonas* sp. Ml-3, *Brevibacillus* sp. Ml-5 and *Bordetella* sp. Ml-6 (named CSK + M356) on cellulose degradation; cellulose degradation efficiency was different at varying sequential culture. Accordingly, four culture mixture was better than three and the three was more efficient than two culture mixture in cellulose degradation and biomass production.

Although, *C. straminisolvens* CSK1 had cellulose-degrading capability under anaerobic conditions, it did not grow under the conditions used for the original microflora, that is, aerobic static conditions (Kato et al., 2004). Likewise, Liesack et al. (2000) reported that anaerobic cellulolytic Clostridia and aerobic bacteria are often simultaneously detected at various sites where cellulose degradation occurs, such as rice paddy soils. In a mixed-culture consisting of *C. straminisolvens* CSK1 and all the aerobic isolates, cellulose degradation occurred under aerobic static conditions, while it did not in the pure culture of *C. straminisolvens* CSK1 or a mixed-culture of the aerobic isolates only. This indicates that the aerobic isolates enable *C. straminisolvens* CSK1 to grow and degrade cellulose under the aerobic static conditions.

Kato et al. (2004) summarized four reasons for efficient degradation of cellulose by mixed culture of CSK + M356. First, the aerobic bacteria supply anaerobic environment, which is an essential condition for growth of *C. straminisolvens* CSK1. The aerobic bacteria would consume oxygen by utilizing substrates contained in yeast extract and peptone, such as peptides and amino acids. Second, the aerobic isolates scavenge metabolites derived from cellulose, which otherwise deteriorate cellulolytic activity. Water soluble cellooligosaccharides, especially cellobiose, are known to repress cellulose degradation by cellulolytic Clostridia. The addition of the aerobic bacteria reduced the concentration of cellooligosaccharides in the culture solution. Third, the aerobic isolates neutralize the pH of the culture solution. It had been shown that the optimum initial pH for growth and cellulose degradation of *C. straminisolvens* CSK1 was 7.5, and little cellulose degradation occurred under pH 6.0. During cellulose degradation by CSK + M356, although the pH value dropped to below 6, it returned to and remained around 7. Although it is not accurately clear how the aerobic bacteria neutralize the pH value, acetic acid consumption would be one of the factors. In addition to the three functions mentioned above, another possible function for strengthening the cellulose degradation in the mixed-cultures might be stimulation of growth of other species by excretion of low-molecular weight compounds. Because of synergistic and better enzyme activity and hence better substrate scarification in coculture, more biomass and protein was obtained. When Eyini et al. (2002) compared protein productivity by the cellulolytic fungi, *T. viride* (MTCC 800), *Chaetomium globosum* and *Aspergillus terreus* in co-culture fermentations of cashew nut bran with monoculture, they found that co-cultures were more effective in substrate saccharification, which ranged between 85-88% as compared to the 62-67% saccharification shown by the monocultures. Maximum saccharification was induced by *T. viride* and *C. globosum* co-culture resulting in the highest 34% release of reducing sugars. As a result of better saccharification, the maximum biomass protein (16.4%) and the highest protein productivity (0.58%) were shown by *T. viride* and *A. terreus* co-culture (Eyini et al., 2002). With similar reason, Duenas et al. (1995) pointed out that the mixed culture in solid state fermentation (SSF) enriched the protein content of bagasse by 13%, converted 46% of its cellulose and hemicellulose into fermentable sugars, and produced the main enzymes for cellulose hydrolysis in optimal proportions with respectable volumetric productivity. In comparison, single culture SSF with *T. reesei* only resulted in 5% protein enrichment, a 32% conversion of cellulose

and hemicellulose, an enzyme complex lacking the critical β-glucosidase components, and a much lower overall volumetric productivity (Duenas et al., 1995).

Starch degradation

Highest level of total digestibility (810.95%) was recorded in potato starch by culture filtrate from mixed culture of *A. niger* and *S. cerevisiae* without mineral supplementation than the monocultures (Abu et al., 2005). More ethanol was produced when amylolytic yeast (*Saccharomyces diastaticus* and *Endomycopsis capsularis*) cocultured with *Saccharomyces cerevisiae* 21 than their respective monocultures due to fast starch saccharification by amylolytic fungi (Verma et al., 2000). Although biomass production decreased, amylolytic activity rate and amount of starch utilization increased several-fold in coculture (*A. niger* and *S. cerevisiae*) versus the monoculture due to the synergistic metabolic interactions between the species (Abouzied and Reddy, 1986). Here, the reason for biomass production decrement should be investigated further. However, it might be the conversion of the carbon source to alcohol or other metabolic products that are not involved in growth.

EFFICIENT CARBON SOURCE UTILIZATION

Cheapest agricultural residues are used as substrates, basically as carbon source, for SCP production by microorganisms. Among these agricultural residues, apple pomace is the residue left after the extraction of juice from the apples. From an animal nutrition point of view, apple pomace is not a suitable feed as it is deficient in digestible protein (Rumsey, 1978). Growth of yeast on the apple pomace increases protein and vitamin contents (Hang, 1988). However, the low level of fermentable sugars limits protein enrichment of the pomace by yeasts; a major portion of the pomace comprises lignocelluloses. Co-culture of cellulolytic moulds *(A. niger* and *T. viridae)* and yeasts (*S. cerevisiae* NCIM 3261, *Candida tropicalis* "NCIM 3119 and *Candida utilis* NCIM 2353) enrich protein in apple pomace (Bhalla and Joshi, 1994) since adequate amount of fermentable sugars are obtained from cellulolytic mould partner. In the co-cultures, the mould hydrolyses the cellulose or hemicellulose component of the pomace by secreting extracellular enzymes (cellulases and xylanases) and the yeast then uses the sugar released. This results in better utilization of the substrate than either microorganism achieved independently (Bhalla and Joshi, 1994).

The use of *Candida kefyr* LY496 alone growing on whey as a source of SCP is disadvantageous because this represents an energetically less efficient method of lactose utilization since ethanol carried out of the reactor in the exit gas stream represents a potential loss of carbon source. In co-culture of *C. kefyr* LY496 and *Candida valida* LY497, no ethanol was detected (Carlotti et al., 1991) because it was used as a substrate by *C, valida* LY497. Hence the carbon source is efficiently utilized.

Several reasons for the efficiencies of nutrient utilization are investigated. Firstly, co-cultures are more efficient than monoculture for enzyme productions that are useful for substrate scarification. For example, in a mixed culture of *T. reesei* LM-UC4E1 mutant and *A. niger* ATCC 10864 with inorganic N-source, 10% more biomass, but 63% more cellulase, 85% more endoglucanase and 147% more β-glucosidase was produced than in single culture (Gutierrez-Correa et al.. 1999). In view of the fact that co-culturing helped enzyme production more than growth, it appeared that synergistic interactions not directly related to growth were responsible for increased enzyme production (Gutierrez-Correa et al., 1999).

FILL SUBSTRATE UTILIZATION GAP

Even though microbes are nutritionally versatile, one microbe cannot utilize all substrates available. However, metabolic product of one microbe could be a substrate for other(s). For instance, *Candida valida* LY497 cannot utilize lactose present in whey. However, it uses alcohol produced by *C. kefyr* LY496 in the co-culture as a substrate (Carlotti et al.. 1991). Hence, *C. valida* LY497 growth in lactose is indirectly possible in co-culture with *C. kefyr* LY496. *S. cerevisiae* cannot utilize starch (Shafiee et al., 2005). Nevertheless, it is greatly important for SCP production when it is compared with *Cryptococcus aerius.* That is why Shafiee et al. (2005) has grown *S. cerevisiae* with *C. aerius* on wheat starch for microbial protein production and the biomass increased by 10%.

A mutualistic symbiotic relationship between *Cellulomonas* and *Pseudomonas* was demonstrated to take place during the mixed growth on bagasse pith; the *Cellulomonas* supplying the carbon source (glucose produced from bagasse degradation) to the *Pseudomonas,* and the latter producing the vitamin supplements necessary for the *Cellulomonas* growth, allowing the growth of the mixed culture in a minimal medium, without any growth factor supplement (Rodriguez and Gallardo, 1993). As a result, high biomass production (19.4 g/L) was achieved on batch culture using this method (Rodriguez and Gallardo, 1993).

REMOVAL OF BYPRODUCT INHIBITION

Microorganisms may produce metabolic products that are inhibitory to the microbes or to others when they grow especially during stationary or death phase. *Kluyveromyces, Candida* and *Trichosporon,* which are involved in SCP production, are capable of metabolizing lactose (Fleet, 1990; Fleet and Main, 1987; Galvez et al.,

1990). However, it has been observed that in the air limited cultures of *Kluyveromyces fragilis,* a change in the cellular metabolism from oxidative to a mixed oxidative-fermentative state can occur. These changes could result in production of by-metabolic products such as alcohol, aldehydes, esters, etc., which reduce the yield of biomass on whey (Beausejour et al., 1981; Moresi et al., 1989; Pigache et al., 1992). It has been shown that a co-culture of *Kluyveromyces* strains and *S. cerevisiae* could overcome these undesired effects (Pigache et al., 1992). Since the *S. cerevisiae* could not grow in lactose medium, it might have consumed some of the extracellular metabolites produced during the growth of *Kluyveromyces* species.

Similarly, Cristiani-Urbina et al. (1997) reported that intermediate compounds such as ethanol, esters etc., formed during aerobic growth of *K. lactis* and *K. marxianus* could reduce the yeast biomass yields. Correspondingly, higher cell mass was produced in monoculture than in coculture (*A. niger* and *S. cerevisiae*), suggesting that substantially more carbon is used for cell production in monoculture, whereas in the coculture most of the substrate carbon is utilized for ethanol production; *S. cerevisiae* was the dominant organism in coculture (Abouzied and Reddy, 1986). These intermediate compounds can be metabolized by some other yeast strains. In Carlotti et al. (1991) study, the ethanol produced by *C. kefyr* LY496 from lactose in whey was utilized by its co-culture *C.valida* LY497. That is why no ethanol was detected in *C. kefyr* LY496 and *C. valida* LY497 co-culture.

The mixed culture of *Lactobacillus kefranofaciens* and *S. cerevisiae* increased the productivity of the lactic acid bacterium and kefiran production (Cheirsilp et al., 2003). Cheirsilp et al. (2003) reasoned out that the yeast assimilate the lactic acid produced by *L. kefranofaciens* and hence the yeast make suitable environment for the lactic acid bacterium. In addition, since it is well known that the yeast possess catalase activity, one possibility is that yeast might reduce the amount of hydrogen peroxide, which is a growth inhibitor of lactic acid bacteria, in mixed culture (Chang et al., 1997).

The major problem in vitamin B12 production using *Propionibacterium*, a bacterium used in SCP production, is the growth inhibition of the cell due to the accumulation of inhibitory metabolites such as propionic acid and acetic acid (Miyano et al., 2000). The propionic acid concentration can be lowered by introducing a mixed culture of *Propionibacterium freudenreichii* and *Ralstonia eutropha,* noting that the latter microorganism can assimilate propionic acid and acetate under aerobic condition (Miyano et al., 2000).

BIOMASS PRODUCTION UPGRADING

In all SCP production, maximizing biomass must be given more emphasis since SCP is produced from microbes. Among different methods, coculturing is one. Accordingly, highest biomass yields were obtained from mixed cultures than monocultures (Carlotti et al., 1991; Ghanem, 1992; Moeini et al., 2004; Shafiee et al., 2005). The efficiency of whey conversion to biomass using *C. kefyr* LY496 and *C. valida* LY497 co-culture was increased by 20% as compared with pure culture of *Candida kefyr* LY496 (Carlotti et al. 1991). Ghanem (1992) found out that a mixed culture of *T. reesei* and *K. marxianus* was found to be more efficient for single cell protein production (51%) from beet pulp than a monoculture of *T. reesei* (49%), Similarly, biomass production increased from 10.02 to 11.22 g/L using mixed culture of *Cryptococcus aerius* and *S. cerevisiae* (Shafiee et al., 2005).

Co-cultures increase biomass productivity by either efficient utilization of substrate or removal of inhibitory by products. Cristiani-Urbina et al. (2000) suggested that the ability of the mixed culture to use several sources of carbon simultaneously might be the main reason for increment of biomass. In addition, when two strains of amylolytic fungi, *A. foetidus* MTCC 508 and *A. niger* ITCC 2012, were assessed for amylolytic activity on a quantitative and qualitative basis in potato chips industry waste, it was found that more enzymes are produced in co-culture than monoculture. This results in better saccharification of substrates. Therefore, more biomass was found in mixed culture (4.55g L^{-1}) than *A. foetidus* (2.4 g L^{-1}) and *A. niger* (2.85 g L^{-1}) monocultures (Mishra et al., 2004). Coculture of *Monascus* cells with a *S. cerevisiae* culture filtrate stimulate reproduction followed by cell proliferation (Suh and Shin, 2000) and raising of biomass. According to Suh and Shin (2000), this is because of the protein kinase C (PKC) since PKC, which is produced by *S. cerevisiae* in co-culture, has a profound effect on cell proliferation and differentiation.

NUTRIENT ENRICHMENT OF SCP

Fermentation is one method of to enhance nutrient content of feed through the biosynthesis of vitamins, essential amino acids, and proteins, by improving protein quality and fiber digestibility (Oboh, 2006). Among different kinds of fermentations, mixed culture fermentation are currently employed to increase nutritional value.

Protein enrichment

Growth of yeast on the agriculture residue increases protein and vitamin contents (Villas-Boas et al., 2002). The low level of fermentable sugars in rape straw for protein enrichment by yeasts was solved using co-culture of *Ganoderma lucidum* and *Candida utilis* (Ke et al., 2011). *G. lucidum* and *C. utilis* produced more protein (16.23%) than monoculture, *G. lucidum,* (8.75 %) when *C. utilis* is added after 7 day fermentation of *G. lucidum.* In the co-cultures, the white rot fungi, *G. lucidum* hydrolyses the cellulose or hemi-cellulose

component of the rape straw by secreting extracellular enzymes (cellulases and xylanases) and the yeast then uses the sugar released (Ke et al., 2011) and produce protein. In the same manner, Oboh (2006) demonstrated that the low protein content of cassava peel was enriched by mixed culture of *S. cerevisiae* and *Lactobacillus* spp. using solid media fermentation techniques.

According to Bhalla and Joshi (1994), the co-culture of *C*. *utilis* and *A. niger* proved to be the best combination in increasing protein content of dried pomace to 20% under SSF conditions. The higher yield of protein from the *C. utilis* and *A. niger* combination probably results from the enzymatic hydrolysis of the lignocellulosic component of the pomace by the *Aspergillus* releasing hexoses and pentoses which *Candida* spp. can efficiently metabolize. *S. cerevisiae,* however, uses only hexoses and consequently is less efficient than the *Candida* spp. Similarly, the protein yield was increased from 49.3 to 54% using co-culture of *T. reesei* and *K. marxianus* on beet pulp wastes (Ghanem, 1992). Shafiee et al. (2005) pointed out that the protein content of the biomass increased from 27 (monoculture of *Cryptococcus aerius)* to 44.7% (mixed culture of C. *aerius* and *S. cerevisiae)* using wheat starch substrate. In the similar study, the amylolytic activity and starch degradation rate increased several fold in co-culture as compared to the monoculture due to synergetic metabolic interaction between the two species even though *S. cerevisiae* is nonamylolytic. Surprisingly, the crude protein increased from 11.3 to 54.5% with a sequential mixed culture of *C. utilis* and *Brevibacterium lactofermentum* on mixed substrates (Rajoka et al., 2011).

Sharma et al*.* (2006) showed that coculture with three cultures gave more protein than coculture with two cultures. The coculture *of Saccharomyces* sp. No. 12 + *Phanerochaete chrysosporium* and *Saccharomyces* sp. No. 12 + *Pleorotus sajor-caju* produce 4.55 and 4.025% crude protein which is more than their respective monocultures. When *Azotobacter chroococcum,* a free living nitrogen fixer, is added to *Saccharomyces* sp. No. 12 + *P. chrysosporium* and *Saccharomyces* sp. No. 12 + *P. sajor-caju,* the crude protein content of SCP increased to 5.075 and 4.55% respectively (Sharma et al., 2006). In addition, they also demonstrated that sequential culture order had great impact on amount of crude protein present in SCP. For instance, the crude protein content of *Saccharomyces* sp. No. 12 + *A. chroococcum* (added 3rd day) + {*P. sajor-caju* + *P. chrysosporium*} (added 6th day) combination and *Saccharomyces* sp. No. 12 + {*P. sajor-caju* + *P. chrysosporium*}(added 3rd day) + *A. chroococcum* (added 6th day) combination was 18.38 and 7.7%, respectively (Sharma et al., 2006).

Amino acid enrichment

When amino acid profiles of the mixed culture (*T. reesei* and *K. marxianus*) was compared with that of monoculture (*T. reesei*)*,* content of some amino acids (leucine, phenylalanine, threonine, valine, aspartic, glutamic and proline) were higher in mixed culture than in monoculture while some others (crystine, methionine, tyrosine, glycine, histidine, and serine) were comparable (Ghanem, 1992). Ghanem (1992) also investigated that amino acid contents are higher than FAO standards except lysine and methionine in co-culture than monoculture.

Lipid enrichment

Monascus sp.JlOl cells co-cultured with a *S. cerevisiae culture* filtrate contained approximately four times more total lipids (mainly linoleic and oleic acid) than *Monascus* cells without co-culture (Suh and Shin, 2000).

Vitamin enrichment

Bacillus firmus AZ-78B and *Streptomyces halstedii* AZ- 8 A mixed culture was found to produce significantly higher yield of the vitamin B12 by growing on medium used for microbial growth under solid state fermentation conditions (SSF) containing agricultural wastes (sugarcane bagasse, wheat straw, rice straw, bean straw and cotton stalks) supplemented with the mineral salts (Atta et al., 2008). Likewise, vitamin B12 production using *Propionibacterium* was done more by coculturing with *Ralstonia eutropha* (Miyano et al., 2000). Cocultures fermentation of *Bacillus* sp. B4 and *Klebsiella* sp.KB2 could improve the quality of Thua-nao by enhancing more soluble protein and vitamin B12 content in the fermented soybean nine times higher than control fermentation with only *Klebsiella* sp.KB2 even though growth of the *Bacillus* sp. B4 and *Klebsiella* sp. KB2 by coculture was lower than that in the monoculture fermentation (Tangjitjaroenkun et al., 2004).

REDUCTION OF PRODUCTION COST

In SCP production, expensive chemicals and pressures were used to treat lignocellulosic substrates. In addition, substrates can be degraded to water soluble and simpler forms using industrial produced and very costly enzymes (Azmi et al., 2010) even though employing enzymes are environmentally friend. Co-culture reduces the cost of enzymes and chemicals required for treatments of lignocellulosic agricultural wastes (Azmi et al., 2009). Chen (2011) demonstrated cost reduction using co-culture *of Pichia stipitis* with *S. cerevisiae* and *Zymomonas mobilis* with cells of *P. stipitis.* However, biological processes can be cost effective and give useful byproducts (Mishra et al., 2004). Similarly, bio-treatment of lignin that protect cellulose and hemicellulose from being decomposed is inexpensive (Tian et al., 2012). Bio-treatment is environmental friendly. The single step

bioconversion from unhydrolyzed cassava starch into ethanol will reduce the cost of enzymes that is normally used in liquefaction and saccharification steps especially on yeast cells (Azmi et al., 2010).

In addition, the maintenance of strict anaerobic conditions for *Clostridium* to grow requires special conditions such as an addition of costly reducing agents into the medium, and flushing with N_2 gas. These factors increase the costs of the fermentation process. However, the cost was minimized by growing *Clostridium botylicum* with aerobic *B. subtilis* in cassava starch since the later removes the oxygen (Tran et al., 2010).

REDUCTION OF FERMENTATION TIME

One problem of producing microbial products and biomass at industry level is the relatively long time it takes to grow microbes as compared to the short time required to manufacture goods. Currently, different trials are being carried out using co-cultures to solve such problems. Co-culture (*Pichia stipitis* with *S. cerevisiae* and *Z. mobilis* with cells of *P. stipitis*) fermentation on lignocellulosic biomass can increase production rate and shorten fermentation time, and it is a promising technology although immature (Chen, 2011). In comparison with fermentation *T. viride* alone, the production time for maximum protein yield was reduced by several days in sequential co culture of *T. viride* and C. *utilis* on wheat straw (Peitersen, 1975). In addition, Shafiee et al. (2005) showed that co-culture of *Cryptococcus aerius* and *S. cerevisiae* fermentation for SCP production using wheat starch as a substrate was 58 h whereas *C. aerius* alone was 78 h.

Paring *S. thermophilus* ST5 with *Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175 resulted in faster fermentation. For instance, *S. thermophilus* ST5 in mixed cultures attains a pH of 4.7 in only 8 h as compared to the 16 h needed for pure cultures of *B. longum* R0175 and 12 h needed by *L. helveticus* R0052 (Champagne et al., 2009). Therefore one major upshot of mixing cultures was reducing the time the bacteria had to grow (Champagne et al., 2010).

STRAIN COMPATIBILITY

Strain compatibility is the determining factor for successful mixed culture fermentation. Therefore, the optimization of compatible mixed culturing of organisms having non-antagonistic behavior should be exploited before they are employed for SCP production (Ikram-ul-Haq et al., 2005). For instance, Hu et al. (2011) showed that the mixed cultivation of *A. niger* and *A. oryzae* produced a little less protein than the individual cultures, while combination of either fungus with *P. chrysosporium* resulted in a small increase in protein production. Only the combination of *A. niger* and *M. grisea* among strains tested at 25°C resulted in a strong increase in protein production (Hu et al., 2011). Similarly, *A. terreus* performed better in co-culture in the presence of *T. viride* (16.4% biomass protein by 88% saccharification) rather than with *Chaetomium globosum* producing 12.4% biomass protein by saccharifying 85% of the substrate in 25 days (Eyini et al., 2002).

Strain compatibility is based on several factors. For instances, when a specified pH is favorable for one partner in coculture it might not be suitable for others. The same might be true for temperature, salt concentrations and other environmental variables (Chen, 2011). As it is discussed above, one may be antagonistic to other by producing harmful chemicals or/and creating not suitable environments. Therefore, strain compatibility should be studied before cocultures are are used for biomass or/and microbial product manufacturing.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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