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# Efficiency of Developed Solid State Bioreactor 'FERMSOSTAT' on Cellulolytic and Xylanase Enzymes Production

(Kecekapan Bioreaktor Keadaan Pepejal 'FERMSOSTAT' yang Dibangunkan ke atas Pengeluaran Enzim Selulolitik dan Xilanase)

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ABSTRACT

*FERMSOSTAT is a developed laboratory scale solid state fermenter. It is a horizontal stirrer drum bioreactor with about 70 L capacities. The fermenter is made of stainless steel which is anti-corrosive and non-toxic to the process organism. The fermenter is equipped with sets of control systems for temperature, agitation, aeration and also outlets for substrate sampling as well as inlets for inoculation and substrate additions. The uniqueness of this FERMSOSTAT system is its ability to carry out* in situ *substrate sterilization and extraction of enzymes at the end of SSF process. Moreover, the mixing system provided by FERMSOSTAT can be performed either full or half mixing as well as forward or reverse mixing. Furthermore, the mixing can be programmed to run at certain agitation rate and time interval during the fermentation process to prevent or reduce damage to the fungus mycelia. FERMSOSTAT is a developed SSF bioreactor and not an improvement of any existing one. The performances of FERMSOSTAT have been evaluated. Under optimum solid state fermentation conditions, about 63.4, 397 and 3.21 U/g of CMCase, xylanase and FPase activities were detected, which were higher compared to the tray system.*

*Keywords: Cellulase; palm kernel cake; solid state fermentation; sugarcane bagasse; xylanase*

ABSTRAK

*FERMOSTAT adalah fermenter keadaan pepejal berskala makmal yang dibangunkan. Ia adalah bioreaktor pengacau gendang mendatar dengan kapasiti kira-kira 70 L. Fermenter ini terdiri daripada keluli tahan karat yang tahan hakis dan tidak toksik terhadap organisma. Fermenter ini dilengkapi dengan sistem kawalan suhu, penggoncangan, pengudaraan dan juga salur keluar untuk persampelan substrat dan salur masuk untuk inokulasi dan penambahan substrat. Keunikan sistem FERMOSTAT ini adalah keupayaan untuk menjalankan pensterilan substrat secara* in situ *dan pengekstrakan enzim pada akhir proses SSF. Selain itu, sistem pencampuran yang dilengkapi oleh FERMSOSTAT boleh melakukan pencampuran secara penuh atau separuh penuh dan juga pencampuran ke depan atau sebaliknya. Tambahan pula, pencampuran boleh diprogramkan untuk melakukan kadar pergolakan yang berlainan dan pada selang masa yang berbeza sepanjang masa proses penapaian untuk mengelak atau mengurangkan kecederaan pada miselium kulat. FERMSOSTAT adalah SSF bioreaktor yang telah dibangunkan dan bukan penambahbaikan daripada yang sedia ada. Prestasi FERMSOSTAT telah dinilai. Dalam keadaan optimum fermentasi keadaan pepejal, kira-kira 63.4, 397 dan 3.21 U/g aktiviti CMCase, xilanase dan FPase telah dikesan dengan nilainya adalah lebih tinggi berbanding dengan sistem dulang.*

*Kata kunci: Fermentasi keadaan pepejal; hampas tebu; isirong kelapa sawit; selulase; xilanase*

## INTRODUCTION

Solid-state fermentation (SSF) can be defined as the fermentation involving moist solids materials in absence (or near absence) of free flowing water (Pandey 1994, 1992; Pandey et al. 2000). The basic principle of SSF is the 'solid substrate bed'. The bed contains moist solids, which are polymeric in nature and insoluble in water are typically the source of carbon, nitrogen, minerals, water and other nutrients for growth and metabolism of the microorganism (Lonsane et al. 1992b). SSF are more applicable for filamentous fungi that grow on the surface of the particle and penetrate through the inter particle spaces into the depth of the bed.

Since ancient, many different types of bioreactors have been traditionally used in SSF processes and they have been given different name by different authors. Furthermore, they have been classified into different groups based on different classification criterions. Generally, they can be broadly classified into tray bioreactor, rotating drum and stirred drum bioreactor, packed bed bioreactor, gas solid fluidized bed bioreactor and stirred aerated bed bioreactor. In fermentation, bioreactor provides control environment for the growth and activity of the microorganism that carried out the biological reactions (Pandey 1991). On the other hand, selection of an appropriate type of bioreactor is also crucial since bioreactor is the 'heart' of the

fermentation process. Furthermore, there are many types of SSF bioreactor which are commercially available and their performances are differs for different type of bioreactors (Mitchell et al. 2006a). However, designing aspects of SSF fermenter has not been given enough attention by the researcher. The important aspect need to be considered during the construction of a bioreactor is the effective regulation of aeration, mixing and heat removal that may affect the yield and quality of the desired product (Perez-Guerra et al. 2003).

Therefore, the purpose of this study was to develop a laboratory scale solid state fermenter, FERMSOSTAT. The performance of this developed solid state fermenter was evaluated on the production studies of cellulases and xylanase enzymes using *Aspergillus niger* USM AI 1 grown on sugarcane bagasse and palm kernel cake as substrates.

## Materials and Methods

### Development and Fabrication of Solid State Fermenter

In this study, a laboratory scale solid state fermenter namely FERMSOSTAT was developed by the authors and fabricated by a local fabricator. The developed solid state fermenter was used through-out the study of SSF process for enzyme production.

### Production of Cellulases and Xylanase Using Fermsostat

*Spore Production and Inoculum Preparation* *Aspergillus niger* USM AI 1 was used in this study for the production of cellulases and xylanase. It was obtained from the Industrial Biotechnology Research Laboratory (IBRL) culture collection, USM Penang. Fungus spore was obtained by growing the culture on Potato Dextrose Agar (Amresco, USA) under room temperature in medium bottles (Lee et al. 2011). The spore was harvested after 4-5 days of cultivation with 0.1% (w/v) Tween 80 (Smits et al. 1996). The spore concentration was estimated by direct microscopic counting using hemocytometer (Raimbault & Alazard 1980). The spore suspension at $1\times10^8$ spore/mL and volume of 20% (v/w) was mixed with the prior autoclaved growth medium. This medium was used as inoculum for the SSF process.

*Substrates Preparation and Sterilization* A mixture of sugarcane bagasse (diameter ≤2 mm) and palm kernel cake (diameter ≤0.5 mm) at 1:1 ratio was used as substrates for cellulases and xylanase production. The substrates were thoroughly mixed by hand prior to transfer into the FERMSOSTAT through the substrate port. The substrate was sterilized *in situ* by hot air sterilization at 130°C for 3 h (Lonsane et al. 1992b). After the sterilization process, the fermenter and substrates were allowed to cool down to ambient temperature before inoculation process were carried out (Lee et al. 2011).

### Medium Composition

The growth medium used in SSF process consisted of mineral salts and trace elements. The composition of the mineral salts composed of (g/L); $NH_4NO_3$, 5.0; $KH_2PO_4$, 5.0; Corn steep liquor, 2.0; NaCl, 1.0; $MgSO_4.7H_2O$, 1.0 and trace elements (mg/L); $FeSO_4.7H_2O$, 5.0; $MnSO_4.4H_2O$, 1.60; $ZnSO_4.7H_2O$, 3.45; $CoCl_2$, 2.0. The pH was adjusted to 7.0 prior to autoclave (Pang et al. 2006).

### Inoculation Process

During the inoculation process, air/water pressure type of spraying nozzles was used to spray the spore suspension and growth medium over the sterilized substrate. In addition, continue mixing of substrate by impeller was carried out during the inoculation. The air supplied was discontinued after all the spore suspension was spray on the substrates (Lee et al. 2011).

### Sampling Process and Enzymes Extraction

The SSF process was carried out for 6 days for enzymes production. About 10 g of substrate was sampling out from each of the sampling port every 24 h interval. Prior to the sampling process, the substrate was mixed for 5 min. The sample was used to determine the enzyme activities and glucosamine content. The enzyme was extracted by mixing the fermented substrates with distilled water containing 0.1% (w/v) Tween 80 (Aikat & Bhattacharyya 2000). The mixture was stand still for 2 h under room temperature with 5 min of mixing every half hour interval. Thereafter, the solid residue was separated from the enzymic solution by filtration through Whatman No.1 filter paper.

### Determination of Cellulases and Xylanase Activities

Cellulase activity was determined based on the carboxymethyl cellulase (CMC)-activity using carboxymethyl cellulose as substrate. The assay method was as described by Gessesse and Gashaw (1999). The enzyme reaction was terminated by adding 3 mL of dinitrosalicylic acid (DNS) reagent followed by boiling for 5 min. The released sugar was measured spectrophotometrically at 575 nm using glucose as standard. Xylanase activity was assay as described by Gessesse and Gashe (1997). The experiment was performed as described in cellulase assay except 1% (w/v) of oat spelt xylan (Fluka, USA) was used as substrate. While, xylose was used as standard to determine the xylanase activity. The cellulase activity can also measure as Filter Paper Activity Unit (FPase). A 1 × 6 cm strip of Whatman No.1 filter paper was added to a total volume of 1.5 mL of culture filtrate and 50 mM citrate buffer (pH4.5). The samples were incubated for 1 h at 60°C. The hydrolysis was terminated by the addition of 3 mL of DNS solution, followed by boiling for 5 min. After cooling, 20 mL of distilled water were added and the absorbance was measured at 540 nm using glucose as standard (Ghose 1987).

One unit of enzyme activity was defined as the amount of enzymes, which catalyzes the liberation of 1 µmol of reducing sugar per min under respective specified assayed condition. Enzyme activity were express as unit (U) per g of fermented substrate, while enzyme productivity was express as unit (U) per mg of glucosamine content of the fungus growth. All assayed were carried out in triplicates and the results were presented as mean of the triplicates experiments.

### DETERMINATION OF FUNGAL GROWTH

The growth of *A. niger* USM AI 1 was examined by determined the glucosamine content of the fungus. Glucosamine content was determined as described by Swift (1972). The glucosamine was measured spectrophotometrically at 530 nm using glucosamine as standard.

## RESULTS AND DISCUSSION

### DEVELOPMENT AND FABRICATION OF A SOLID STATE FERMENTER

Since ancient, many different types of bioreactors have been traditionally used in SSF processes and they have been given different name by different authors. Furthermore, they have been classified into different groups based on different classification criterions. It is well known that the static reactors have some limitations compared to the agitated fermenters (Lonsane et al. 1992a). However, the damage to the mycelia cause by attrition and the breakage of the attachment of mycelia to the solid substrate particles in agitated SSF reactors are also well documented (Lonsane et al. 1992a; 1985). Therefore, a combination of best design features of static and agitated reactors thus may lead to high fermentation productivity. Hence, such an attempt was made while designing and developing FERMSOSTAT. FERMSOSTAT is a developed laboratory scale solid state fermenter. It is a horizontal stirrer drum fermenter and according to Mitchell et al. (2006b; 2003b) classification criterions, it was classified into group III. The bed in this bioreactor group is either intermittently or continuously mixed and the air is circulated around the bed but do not forcefully blown through the bed. Figures 1 and 2 show the photography of FERMSOSTAT equipped with aeration/cooling and inoculation system and schematic diagram, respectively. The fermenter is made of stainless steel which is anti-corrosive and non-toxic to the process organism. The fermenter is equipped with sets of control systems for temperature, agitation, aeration and also outlets for substrate sampling as well as inlets for inoculation and substrate addition. The uniqueness of this FERMSOSTAT system is its ability to carry out *in situ* substrate sterilization and extraction of enzymes at the end of the SSF process. FERMSOSTAT is a not an improvement of any existing one.

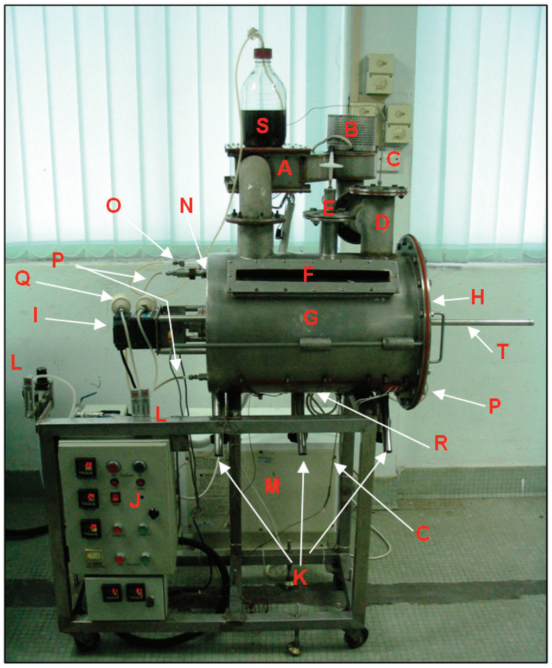



FIGURE 1. FERMSOSTAT® with complete fermentation system

### FERMENTOR VESSEL

A horizontal drum with dimensions of 40 cm internal diameter and 56 cm long, made of stainless steel with a capacity around 70 L was used as the fermentor body (Figure 2: label G). The fermentor vessel consisted of a viewing glass (40 cm long × 3.5 cm width) for facilitate contamination monitoring (Figure 2: label F). Two thermocouples were incorporated into the fermentor body to monitor the temperature profile inside the substrate bed and at headspace (Figure 2: label C) throughout the fermentation process. The opening (door) with 50 cm diameter was located on the right side of the fermentor vessel (Figure 2: label H) to facilitate products/biomass unloading and cleaning of the fermentor vessel after fermentation process. In addition, flat rubber was incorporated at all of the opening of the fermentor to ensure closely fit of the fermenter unit.

### STERILIZATION SYSTEM

FERMSOSTAT is equipped with *in situ* sterilization system. Thus, it is easy to control any contamination during the fermentation process. The solid substrate and fermenter vessel can be sterilized together using hot air sterilization at 130°C for 3 h. A 200 mesh sieve was located in between the fan (Figure 2: label B) and substrate port (Figure 2: label D) to prevent the entry of any substrate into the fan and heater. The sterilization process can be programmed to run at any desired temperature and time. Heating process will automatically cut off at the end of the sterilization process. In the sterilization process, hot air was obtained from the heating coils located on the top left of the fermentor vessel with the help of internal fan that generate air circulated around the fermenter to sterilize the solid substrate and

fermentor. Heater was set at higher temperature (200°C) due to heat lost in the fermentor vessel during the sterilization process. This can be seen by the temperature of hot air detected by the thermocouple located at the headspace was around 140°C and even less at the bottom of the fermenter vessel (<100°C). This shows that hot air sterilization alone may not be enough to sterilize the solid substrate and fermentor. Therefore, one piece of heating plate (Figure 2: label R) was added/paste at the bottom of the fermentor vessel and heated at 130°C to ensure better sterilization especially at the bottom of the fermentor vessel and also the solid substrate.

## MIXING SYSTEM

Mixing is one of the important parameters in SSF process, since it ensures homogeneity in terms of temperature and gaseous environment promotes heat transfer efficiency and facilitate uniform distribution of nutrient added during the fermentation process (Lonsane et al. 1992a). On the other hand, mixing may have some disadvantages such as adverse effect on fungus growth but in many cases their advantages are always outweigh the adverse effect (Pandey et al. 2000a). Figure 3 shows the photography of mixing system provided in FERMSOSTAT. Impeller shaft with dimensions of 100 cm long × 2 cm diameter was located along the central axis of the fermenter vessel (Figure 3(Z)). One end of the impeller shaft was mounted by a speed control motor. In addition, 54 cm long × 38 cm width × 0.5 cm thick rectangular paddle was mounted at the central axis (Figure 3(Z): Label I). One flat rubber was fitted at each end of the rectangular paddle to facilitate removal of any substrate particles that attached to the internal wall of the fermenter vessel during the agitation process. Four paddles mixer (Figure 3(Z): Label H) with dimensions of 18 cm long × 2.5 cm width × 0.5 cm thick were placed at an equal distance (±8 cm) and 90° angle relative to each other were located in between the big rectangular paddle. The central axis was driven by a speed control motor, which can control the mixing rate varying from 0.5 to 15 rpm (Figure 3(Y): Label C). With the help of two mixing sensors (Figure 3(B): Label B) that located near the speed control motor, the mixing system can be performed either full or half mixing as well as forward or reverse mixing. Furthermore, the mixing can be programmed to run at certain agitation rate and time interval during the fermentation process (Figure 3(Y): Label E and F). This is because the use of intermittent rather than continuous agitation will be more appropriate to prevent damage to the mycelia (if any) and disruption of mycelia attachment to solids especially if the hyphae have no septa (Lee et al. 2015, 2013, 2011).

## AERATION SYSTEM

Temperature control of substrate bed during SSF process is very important since it affects the growth of the microorganism, spore formation and germination as well as desired product formation (Pandey 2003). Heat removal is probably the most crucial factor in SSF processes. Heat transfer is very low due to the poor thermal conductivities or limited heat transfer capacity of the solid substrates used (Perez-Guerra et al. 2003) as well as the low moisture content (Raimbault 1998). The heat removal by convection and conduction is poorer in most of the SSF systems owing to the absence of agitation or aeration system. For these reasons, heat removal has been thought to be an insurmountable problem in large SSF bioreactors of conventional types (Laukevics et al. 1984). The necessity of increasing heat removal was taken into account when elaborated the FERMSOSTAT system. Air supply to the fermenter vessel was drawn from air compressor through pressure regulator (Figure 2: Label M). The air was subjected to deoiling in an oil separator and dust removal by dust filter. The air was sterilized by pass through the 0.2 um sterilized air filters. FERMSOSTAT was equipped with two air entry points (0.5 cm in diameter tube) to improve heat removal. One air entry point was located 3.5 cm from the bottom left side of the fermenter vessel (Figure 2: Label P), while the other was located 8.5 cm from the bottom of the door opening. The air supplied to the fermenter vessel was initially split into 2 aeration tubes that were then individually control by the control valve and flow meter before enter into the fermentor vessel. The highest air flow rate that can be supplied by the compressor air was around 40 L/min. An air outlet/exhaust (Figure 2: Label E) was situated on top of the fermenter vessel and the air was prior filtered by air filter before released to the environment.

## INOCULATION SYSTEM

In most of any fermentation process, high ratio of inoculums was generally used for the production of biomass and desired secondary products with the aim of increasing process productivity in term of obtaining the desired level of final product in a shorter period of time. Similarly, most of the SSF processes also used higher ratio of inoculum. This was not only intent to increase process productivity but the main purpose is to prevent contamination during fermentation process (Lonsane et al. 1992a). In this study, spore suspension at $1\times10^8$ spore/mL and volume of 20% (v/w) was mixed with the prior autoclaved growth medium. This medium was used as inoculum for SSF process. The inoculation process was carried out using Air/water pressure type of spraying nozzle (5 mm diameter) (Figure 2: Label N, O) to spray the spore suspension (Figure 2: Label S) and growth medium over the solid substrate. Continue agitation was carried out during the inoculation process because industrial spraying nozzle usually cannot spray evenly over the whole surface of the bed. The spraying nozzle was located at the top left side of the fermenter vessel (Figure 2: Label N). Sterilized air drawn from the air compressor was entered to the air inlet point at the spraying nozzle. This process will create a pressure drop condition at inoculum inlet point (Figure 2: Label O), which consequently will draw the inoculum solution from the bottle and enter to the fermenter vessel

through the inoculum inlet point. The air supplied was discontinued after all the inoculum had been transferred into the fermenter. However, mixing was continued for another 15 min in order to allow the absorption of the inoculum by the solid substrate.

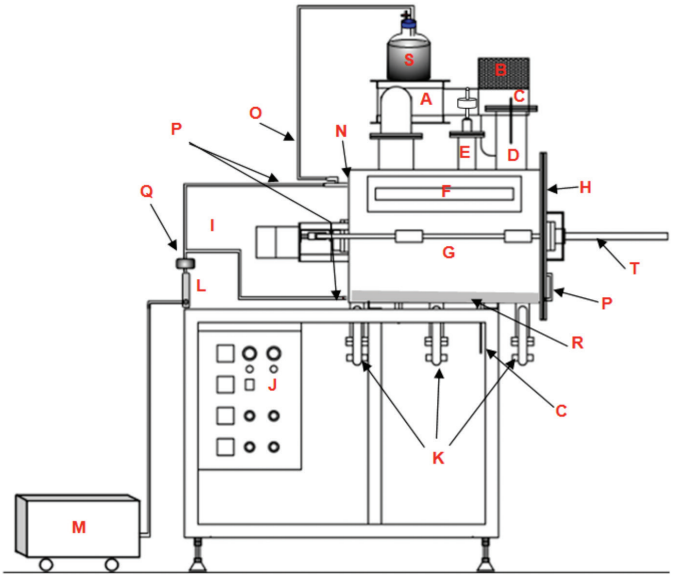
## COMPARISON OF FERMSOSTAT WITH OTHER GROUPS OF FERMENTER

FERMSOSTAT, a horizontal stirrer drum fermenter is a newly developed of laboratory scale SSF fermenter and not an improvement of any existing one. According to Mitchell et al. (2006b, 2003b) classification criteria, FERMSOSTAT is classified into group III together with rotating drum bioreactor in which, the bed in this bioreactor group is either intermittently or continuously mixed and the air is circulated around the bed but do not forcefully blown through the bed. In general, mixing efficiency in stirrer drum bioreactor is more efficient than rotating drum bioreactor (Durand 2003). Temperature gradient and low heat transfer efficiency are common problems in most of the SSF process and these problems are taken into account when designing FERMSOSTAT. The location of air inlet and outlet of aeration system may have great influence on the air flow pattern at the headspace of the bioreactor. Air inlet and outlet for most of the designed bioreactor is located near the headspace (Mitchell et al. 2006a). However, in FERMSOSTAT two air inlet points are located near the bottom end of the fermenter vessel and air outlet located on top of fermenter vessel are believed to be able to increase the efficiency of heat removal during the course of the fermentation process.

As well documented, static reactors have some limitations compared to any agitated fermenters (Lonsane et al. 1992a). However, Lonsane et al. (1992a) reported adverse effects on substrate particles, disruption of fungal attachment to the solids and damage to fungal mycelia due to shear forces in agitated SSF systems. Nevertheless, no adverse effects of agitation in the production of gibberellic acid (Kumar & Lonsane 1988, 1987) have been reported. Moreover, the agitation may prevent formation of substrate agglomeration depending on the nature of the solids used (Lonsane et al. 1992a). Therefore, a combination of design features of static and agitated reactors has been attempt while designing and developing of FERMSOSTAT. Intermittent mixing and half mixing as well as forward and reverse mixing that can be performed by FERMSOSTAT are to reduce or minimize damage to the mycelia (if any) and disruption of mycelia attachment to solids.

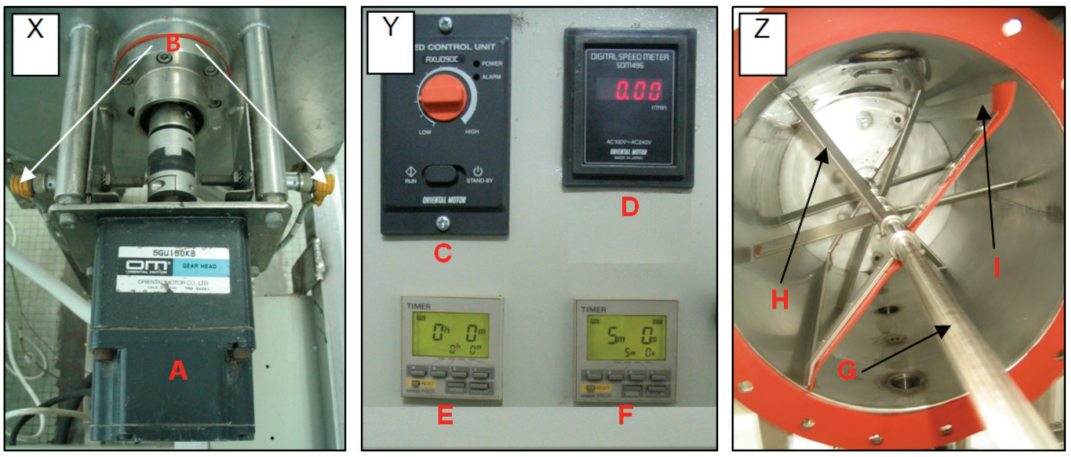
On the other hand, the possible drawback of stirrer drum bioreactor (Include FERMSOSTAT) is insufficient heat removal at larger scale due to heat removal through the fermenter wall becomes inadequate as the volume increases (Nagel et al. 2001). In addition, for stirrer drum and rotating drum bioreactor, not whole fermenter vessel can be fill with substrate and the suitable amount of substrate that can be loaded is mainly dependent on the 'fractional filling' of the drum. Indeed, in rotating drum bioreactor agglomeration of substrate particles can occur, which would increase the problem in regulating the temperature of the substrate bed. Not only that, if water jacket is presence and it would increase the weight of the bioreactor to be rotated and thus increase the overall power consumption (Lonsane et al. 1985).

Tray bioreactor consists of a chamber in which air with controlled temperature and relative humidity is circulated around a number of trays. Each tray contains a thin layer of substrate. Mostly manual mixing by hand is




A: Heater; B: Fan; C: Thermocouple; D: Substrate Port; E: Exhaust; F: Viewing Glass; G: Fermentor vessel; H: Opening; I: Speed Control Motor; J: Control Panel; K: Sampling Port; L: Flow meter; M: Air Compressor; N: Spraying Nozzle; O: Inoculum inlet; P: Air Inlet; Q; Air filter, R: Silicon rubber heater; S: Inoculum; T: Impeller Shaft

FIGURE 2. Schematic diagram of FERMSOSTAT® with complete fermentation system




A: Speed control motor; B: Mixing sensors, C: Speed control unit, D: Digital readout for mixing rate, E: Static incubation time, F: Mixing time, G: Impeller shaft, H: Impeller tips, I: Rectangular paddle

FIGURE 3. Mixing system provided in FERMSOSTAT®. (X) Speed control motor, (Y) Various digital readout and (Z) Impeller

carried out once a day. Scale up in tray fermenter cannot be achieved simply by increasing the substrate bed height in tray due to heat and mass transfer limitations and over-heating problem in trays system has been well documented. Scale up can only be done by increasing tray size (area) or number of tray used. Rathbun and Shuler (1983) found out that during tempeh fermentation the temperature gradients as high as 3°C $cm^{-1}$ and $O_2$ gradients of 1.7% (v/v) have been detected. However, Chen et al. (2005) have successfully developed a newly and effective bioreactor which can enhance the heat transfer and effectively control the temperature variation in SSF process. By using two dynamic changes of air during fermentation developed by the authors, the maximum temperature difference at a height bed of 9.0 cm was only 0.12°C $cm^{-1}$ compared with traditional tray SSF system where the temperature gradient was more than 3°C $cm^{-1}$ at a bed height of 6.5 cm. On the other hand, the modeling work over the last 10 years has shown how the mass and heat transfer limitations interact to limit tray performance. The thickness of the substrate bed must not more than few centimeters. Tray system is ideal for relatively low volume products and larger scale processes are labor intensive and not economical. This was due to the fact that automation of all the different steps in tray system is extremely difficult. Therefore, it was often used in countries where labor costs are low. In addition, tray system is not suitable for pilot scale process because it is not practical to handle thousands of trays and thus others type of fermenter must be considered (Mitchell et al. 2000b).

A packed bed bioreactor typically consists of substrate packed into a vertical column with substrate supported on a perforated base plate. Conditioned air is blown upward from the bottom of the column. These types of bioreactors have been used to produce various types of products such as enzymes, ethanol and biopesticides (Hardin 2004). One of the drawback of packed bed bioreactor is water loss due to evaporation from the surface of the substrate bed is impossible to control. In addition, it is impossible to prevent temperature gradient during the fermentation process. Temperature gradients promote evaporation loss of water even if air saturated with water is used to aerate the column. Nevertheless, replenishment of water is not practical within an unmixed bed because it is very hard to distribute the water evenly throughout the bed. Therefore, it is desirable and crucial to minimize water loss, reduce the temperature gradient and increase heat removal in packed bed fermenter (Mitchell et al. 2006c, 2000b). Another important phenomenon in static packed-bed bioreactors is the pressure drop through the column during fermentation, which can affect the ability of the process air to pass through the column. High pressure drops will likely to cause air channeling to occur, which consequently may result in little air to reach other part of the bed. However, this problem can be reduced by agitating the substrate bed but this would possible cause deleterious effects on the fermentation (Hardin 2004; Mitchell et al. 2000b). 'Prophyta' and 'Plafractor™' bioreactors are the recent developed packed bed bioreactors, with the use of a number of thin beds coupled with cooling plates oriented normal to the air flow (Luth & Eiben 1999; Suryanarayan 2003; Suryanarayan & Mazumdar 2000). 'Plafactor™' bioreactor is computer controlled, while 'Prophyta' is used for producing biopesticides in sterile conditions (Durand 2003).

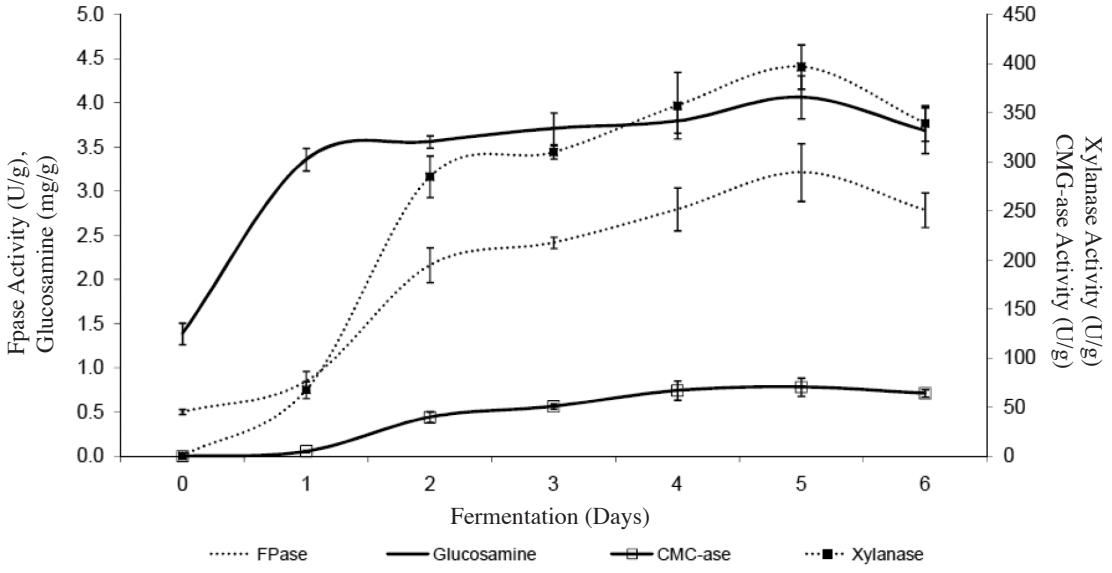
Air solid fluidized bed fermenter is a vertically laid columns bioreactor, in which air is blown upward through the perforated base plate with sufficient velocity to fluidize the substrate bed. A mechanical mixer or a breaker is usually incorporated in order to break any agglomerates. The column must be taller enough and extra space must be available for bed expansion that takes place upon fluidized. The process air can be recycled in order to save the air preparation costs. Kikkoman Corporation had constructed

8000 L working volume of air solid fluidized bed fermenter in 1975 and successfully used the system for production of enzymes by *Aspergillus sojae* using wheat bran as substrate. In addition, Matsuno et al. (1993) has also using this type of bioreactor for enzymes production by fungi. Temperature gradient and metabolic heat removal may not be a problem since high air flow rates is used during the fermentation process. Furthermore, the substrate bed can be well mixed and the mixing action should be relatively gentle when compared to mixing by mechanical agitators. Unlike other types of bioreactor, operating expenses for this reactor are likely to be quite high. Moreover, this type of bioreactor is not suitable used for sticky substrate that tend to agglomerate and the bed will not fluidize. In addition, variations in substrate particles size and shape could lead to substrate particles segregation. Therefore, this type of bioreactor is not suitable for all SSF processes (Hardin 2004; Mitchell et al. 2000b).

## PRODUCTIVITY EVALUATION

Data presented in Figure 4 shows the optimum production of cellulases and xylanase enzymes by *A. niger* USM AI 1 using developed solid state bioreactor FERMSOSTAT. As indicated in the figure, all the enzymes production increased rapidly after first day of fermentation process and reaching maximum level after 4-5 days of fermentation process. Glucosamine profile showed that *A. niger* USM AI 1 growth rapidly after inoculation and reaching maximum level after 5 days of fermentation process with glucosamine content of about 3.86 mg/g fermented substrate. Unlike FPase and xylanase enzymes, slightly declined in CMCase activity was detected after optimum production day. Highest CMCase activity of 63.4 U/g was obtained after 4 day of fermentation process with enzyme productivity of 17.7 U/$mg_G$ glucosamine. On the other hand, as reported by Gao et al. (2008), highest CMCase activity of 581 U/g was produced by *Aspergillus terreus* M11 using corn stover as substrate under the optimum fermentation conditions of 45°C incubation, pH3.0, 80% (w/w) moisture content and 0.8% yeast extract as carbon and nitrogen sources. However, optimum CMCase activity of 25.2 U/g was detected after 3 days of fermentation process using *Trichoderma reesei* Rut C-30 grown on Kinnow pulp and wheat bran at 3:2 (w/w) ratios in Mandel Weber medium (Oberoi et al. 2010).

As also exhibited in Figure 4, xylanase production achieved maximum level after 5 days of fermentation process with enzyme activity and productivity of 397 U/g and 103 U/$mg_G$ glucosamine, respectively. Marginally decreased in xylanase activity was observed after maximum production day shows that xylanase was not a stable enzyme. However, the result obtained was in disagreement to Xu et al. (2008), who obtained optimum xylanase activity of 14637 U/g after 2 days of fermentation process using *A. niger* XY-1 grown on wheat bran in the shake flask. Meanwhile, Considine et al. (1988), obtained about 386 U/g of xylanase activity when *T. reesei* MCG7 was grown on beet pulp at 70% (w/w) moisture content and 27°C. On the other hand, FPase production increased and reaching maximum level after 5 days of fermentation process. Under optimum production day, about 3.21 U/g and 0.83 U/$mg_G$ glucosamine of enzyme activity and productivity were detected, respectively. At the same time, about 13% reduction in enzyme production was detected after the enzyme achieved optimum production day. As reported by Chandra et al. (2007), who obtained maximum level of FPase activity (2.9U/g) after 3 days of fermentation




The SSF process was carried out using 0.5 kg substrate; 70% (w/w) moisture content; 30°C; aeration at 4 L/h•g fermented substrate for 5 min and mixing at 0.5 rpm every 24 h interval for 5 min. Arrow bars indicate means with standard error of three replicates

FIGURE 4. Optimum production of cellulases and xylanase enzymes by *A. niger* USM AI 1 using FERMSOSTAT

process using *A. niger* grown on wheat bran at 50% (w/w) moisture content and incubated at room temperature. At the same time, maximum FPase activity of 6 U/g was detected after 3 days of fermentation process when *Trichoderma wide* SL-1 was grown on Chaff and whet bran at 7:3 (w/w) ratio under optimum conditions of 60% (w/w) moisture content and incubated at 30°C (Tao et al. 1996).

On top of that, the efficiency of enzymes production using FERMSOSTAT has been compared with the tray system. Under optimized SSF conditions using the tray system, the highest CMCase, xylanase and FPase activities obtained were 56.2 g/L, 336 U/g and 2.8 U/g, which were about 11.4%, 16.3% and 21.4% lower compared to FERMSOSTAT, respectively (Lee et al. 2007). These findings indicated that FERMSOSTAT serves its purpose as an SSF fermenter for higher enzyme production.

## Conclusion

Many aspects pertaining to the bioreactor design are yet to be studied in detail. However, the result obtained in this study indicated that the developed solid state fermenter, FERMSOSTAT was able to produce cellulase and xylanase with reasonable good titer, which was higher than those produced with the tray system. Furthermore, the results obtained were comparable with findings reported elsewhere. The ease of operation and simplicity in design as well as reasonable capital cost are the attractive features for FERMSOSTAT. The development of FERMSOSTAT may bring about easier collection of lab scale data that may facilitate future up scaling studies. Thus, FERMSSTAT has great potential in promoting transfer of SSF technologies for industrial exploitation.

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