Growth, substrate consumption, and product formation kinetics of *Phanerochaete chrysosporium* and *Schizophyllum commune* mixed culture under solid-state fermentation of fruit peels

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Received: 27 February 2018 / Accepted: 24 September 2018 / Published online: 28 September 2018
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Abstract

Kinetic analysis of solid-state fermentation (SSF) of fruit peels with *Phanerochaete chrysosporium* and *Schizophyllum commune* mixed culture was studied in flask and 7 kg capacity reactor. Modified Monod kinetic model suggested by Haldane sufficiently described microbial growth with co-efficient of determination (R^2) reaching 0.908 at increased substrate concentration than the classical Monod model ($R^2=0.932$). Leudeking–Piret model adequately described product synthesis in non-growth-dependent manner ($R^2=0.989$), while substrate consumption by *P. chrysosporium* and *S. commune* fungal mixed culture was growth-dependent ($R^2=0.938$). Hanes–Woelf model sufficiently represented α -amylase and cellulase enzymes synthesis ($R^2=0.911$ and 0.988); α -amylase had enzyme maximum velocity ($V_{\max}$) of 25.19 IU/gds/day and rate constant (K_m) of 11.55 IU/gds/day, while cellulase enzyme had $V_{\max}$ of 3.05 IU/gds/day and K_m of 57.47 IU/gds/day. Product yield in the reactor increased to 32.65 mg/g/day compared with 28.15 mg/g/day in shake flask. 2.5 cm media thickness was adequate for product formation within a 6 day SSF in the tray reactor.

Keywords Kinetics · Leudekin–Piret · Monod model · Growth rate · Fermentation

Introduction

Solid-state fermentation (SSF) which is the cultivation of microbial cells in the absence of free flowing water is one of the most adopted methods for the valorization of food and agricultural wastes (Zhang et al. 2013). SSF mimics the natural habitat of fungal cells leading to application in producing value-added products like enzymes, ethanol, animal feed, organic acids, and vitamins (Jamal et al. 2012; Díaz et al. 2012; Lizardi-Jiménez and Hernández-Martínez 2017). SSF technology is robust because of its characteristic low energy requirement, high substrate capacity, low infrastructural input, and potential in solving environmental challenges (Nath and Das 2011; Aggelopoulos et al. 2014).

Phanerochaete chrysosporium (*P. chrysosporium*) is an edible basidiomycete that grows, metabolizes, and consumes virtually all lignocellulosic residues due to its secretion of extracellular enzymes that degrade lignin, cellulose, hemicellulose, and other complex organic compounds found in agricultural wastes (Shi et al. 2014). Lignin peroxidase, manganese peroxidase, and glyoxal oxidase are examples of hydrolytic enzymes secreted by *P. chrysosporium* to depolymerize organic residues and unwind lignin crosslinks (Gad et al. 2010; Shi et al. 2014). Another edible fungi used in this study—*Schizophyllum commune* (*S. commune*) is a less known edible fungus capable of metabolizing complex fruit wastes. *S. commune* is widely consumed in Brazil, India, and Sub-Saharan Africa for protein augmentation of staple foods (Roman 2010). Although there are reports concerning valorization of agricultural wastes through cultivation of *S. commune*, its use is limited to monoculture SSF leaving a wide gap to explore in the mixed culture option (Zhu et al. 2016; Sornlake et al. 2017).

Investigation of growth, substrate consumption, and product formation kinetics of mixed fungal cultures in a batch fermentation is essential for designing and optimizing process conditions (Spier et al. 2009; Song et al. 2010).

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Therefore, the application of suitable batch kinetic models that can describe the growth of fungal mixed culture, substrate utilization, and product formation is vital prior to pilot investigation. Monoculture SSF processes enjoy the application of various kinetic models that describe the fermentation parameters, though the kind and number of applied models depend on products and microbial class (Surendhiran et al. 2014).

In practice, empirical models are frequently used in validating constants of monoculture SSF; hence, their application to mixed fungal fermentation process is scanty (Spier et al. 2009). Application of empirical models will ease determination and validation of constants associated with growth and development of mixed fungal cultures under SSF. Determination of rate constants of fungal growth, enzyme secretion, and protein synthesis in a mixed fungal SSF is a holistic approach to quantitatively estimate extracellular products and their feasibility for pilot scale SSF (Song et al. 2010; Linville et al. 2013).

Kinetic analysis of mixed strains of basidiomycete fungi in SSF vis-à-vis growth rate, substrate consumption, and product formation remained scanty. In the present study, Monod kinetic model and its proposed modification was employed to describe the relationship between biomass growth and substrate consumption in the pre-optimized batch process. Another empirical model that describe product synthesis as a function of substrate consumption with a focus on growth-associated and non-growth-associated constants in the SSF process was included (Song et al. 2010). Hanes–Woolf kinetic model for active enzyme synthesis was used to investigate enzyme kinetics of the batch process (Sethi et al. 2016). Optimum substrate depth and other fermentation parameters were identified.

Materials and methods

Fruits peel collection and media preparation

The growing media comprised banana (*Musa sapientum*) peels (Bp), Pineapple (*Ananas cosmos*) peels (PAp), and Papaya (*Carica papaya*) peels (Pp) collected from fruit processors within Gombak metropolis (Selangor, Malaysia). After washing the peels to remove adhering foreign materials, drying at 60 °C for 48 h in a hot air oven followed immediately to arrest microbial degradation and prevent out-set of milliard reaction. Dried substrates undergo milling to 2 mm particle size with a grinding machine (Model D-79219 Staufen, IKA-WERKE GMBH & Co KG Germany) equipped with customized 2 mm sieve mesh and stored in airtight container. The pulverized peels were mixed (0.35 g Bp, 5.5 g PAp, and 0.15 g Pp), so that the mixture contains 500 mg/g metabolizable sugar, minerals— KH_2PO_4 (1.2 g/l),

CaCl_2 (0.8 g/l), and nitrogen source—peptone (0.8 g/l) were added before moisture content was adjusted to 70.2%.

Microorganisms and inoculum preparation

Two white rot fungi comprising isolated and identified *S. commune* strain with accession number EU530002 (GI: 170280299) and laboratory stock of *P. chrysosporium* (ATCC 20696) were employed for mixed culture fermentation process. *S. commune* was cultivated on malt extract agar (MEA, Merck, Germany) for 7 days at 30 °C in incubator (Memmert), while *P. chrysosporium* was cultivated on potato dextrose agar (PDA, Merck, Germany) for 7 days at 30 °C; each strain was subcultured every fortnight. Inoculum was prepared by adding 15 ml distilled water to fungi colonized plate and scrapped with L-shaped rod to remove spores. Suspended spores were transferred to 250 cm³ sterilized Erlenmeyer flask and stored at 4 °C. 1.25 ml *S. commune* mycelia suspension (0.865 g/kg dry weight) and 1.75 ml of *P. chrysosporium* spore suspension (0.865 g/kg dry weight) mixture (equivalent to 1×10^8 spores/ml) was vortexed prior to inoculation.

Solid-state fermentation (SSF)

SSF in Erlenmeyer flask

The first-stage SSF process comprises 5.96 g substrate (29.8%) and 14.84 g total liquid (70.2%) containing inoculum, KH_2PO_4 (1.2 g/l), and CaCl_2 (0.8 g/l) to maintain 70% moisture content in 250 ml Erlenmeyer flasks of 20 g working volume. The flask content was sterilized at 121 °C for 15 min, cooled to room temperature, and aseptically inoculated. Triplicate flask samples were incubated at 32 °C in Memmert incubator for 7 days.

SSF in a tray reactor

The dimension of the tray reactor made of a microwaveable plastic equipped with airtight reinforced cover was 333 mm × 240 mm × 135 mm with 7 kg capacity (Fig. 1). The cover of the reactor was perforated at eight equidistant points with a diameter of 25 mm to facilitate sterilized static aeration through cotton plug inserted into the 25 mm hole. Substrate holding capacity of the reactor was optimized by one-factor-at-a-time (OFAT) methodology by varying substrate depths (between 2.0 and 4.0 cm); fermentation period and product synthesis; all experimental determinations were in triplicates. The inoculum and mineral composition [KH_2PO_4 (1.2 g/l) and CaCl_2 (0.8 g/l)] of the 7 kg capacity reactor remained unchanged but scaled as per Erlenmeyer flask, while substrate depth changed during optimization. The inoculated tray reactors were incubated in a pre-sterilized controlled environment

Fig. 1 Diagrammatic representation of the tray reactor

chamber (Jiotech) running at 95% humidity, 32 °C, and static airflow.

Kinetic models

Microbial growth-related kinetics

Different kinetic equations (linear, exponential, logistic, and Monod equation) were used to identify batch fermentation constants of fungal biomass growth during batch SSF of fruit peels (Linville et al. 2013). Hence, the specific growth rate (μ) in the growing and death phase of the process was assumed to follow first-order reaction, so the specific growth rate at this stage was calculated; thus:

$$\mu = \ln(x_2/x_1)/(t_2 - t_1), \quad (1)$$

where x_1 and x_2 are the glucosamine content of the cell obtained at time t_1 and t_2 , respectively.

Classical Monod equation empirically fits several types of data adequately and is the most commonly applied unstructured, non-segregated model in characterizing kinetic microbial growth constants in growth-linked substrate utilization. The model suggests that the microbial specific growth rate μ depends on the growth-limiting substrate (S) (Manikandan et al. 2008; Song et al. 2010), such that:

$$dx/dt = \mu x, \quad (2)$$

$$\mu = \mu_{\max} S/(k_s + S), \quad (3)$$

where μ is specific growth rate (day^{-1}), $\mu_{\max}$ is maximum specific growth rate (day^{-1}), K_s is half-saturation constant (mg/g). X is microbial cells measured as glucosamine concentration (mg/g), S and t are initial substrate concentrations (mg/g), and time (day), respectively.

However, several investigators opined that classical Monod model does not fit all the conditions, especially when there is need to account for inhibition due to high substrate concentration. Therefore, another model that could account for substrate inhibition of protein-synthesizing mixed fungi is necessary. The inclusion of substrate inhibition factor in classical Monod equation is important, since the values of the growth kinetic parameters could be difficult to derive from cultures at high substrate concentrations (Nath and Das 2011). Substrate inhibition model according to Andrew's equation adequately described protein synthesis using fruit peels (Bp, PAp and Pp). Andrew's model demonstrated a non-linear relationship between the specific growth rate and substrate concentration (Andrews 1968):

$$\mu = \mu_{\max} S/(k_s + S + (S^2/k_i)), \quad (4)$$

where other parameters remained the same as Eq. 2 above, K_i (mg/g) is inhibition constant at elevated substrate concentration.

Kinetics of product formation

Kinetic model suggested by Luedeking and Piret was originally designed for gluconic acid production using bacterial cells and it asserts that product formation depends on

microbial growth (dX/dt) during the fermentation process in a linear fashion (Nath and Das 2011). The equation has since been applied to characterize product kinetics of monoculture cultivation of filamentous fungi under submerged fermentation (SmF) and SSF (Manikandan et al. 2008; Sethi et al. 2016). The model appeared suitable for identifying kinetic constants of mixed fungi SSF for improved synthesis of protein. The equation is depicted; thus:

$$dP/dt = \alpha dx / (dt + \beta x), \quad (5)$$

where α (mg/g) and β (mg/g) are the Leudeking–Piret equation parameters for growth- and non-growth-associated constants respectively. The substrate consumption on the other hand can be depicted with the same model as:

$$dS/dt = -\gamma dx / (dt - \lambda x), \quad (6)$$

where γ (mg/g) and λ (mg/g) are the Leudeking–Piret equation parameters for growth- and non-growth-associated constants for substrate consumption.

A linearized form of Michealis Menten model, described by Hanes and Wolf, was used to analyze enzyme kinetics of the SSF process.

$$S/V = k_m / (V_{\max} + S/V_{\max}). \quad (8)$$

The equation is linear by the plot of ' S/V ' against ' S '; K_m and $V_{\max}$ were calculated from the slope ($1/V_{\max}$).

Analytical methods (protein, sugar, and carbohydrate assay)

Protein content of dried fermented product was determined in all the cases using folin phenol reagent methodology (Lowry et al. 1951). Ten mg of sample was extracted with 5 ml of 1 N NaOH, allowed to stand for 24 h at room temperature, and centrifuged at 6000 rpm for 15 min, and supernatant was used for the assay. Soluble sugar and total carbohydrate content of the samples were assayed using phenol–sulphuric acid reagent (Dubois et al. 1956). Total soluble sugar was determined by extracting 100 mg of samples with distilled water, filter with Whatman no. 1 filter paper, and 1 ml of the filtrate served as sample for assay. Total carbohydrate was assayed by first hydrolyzing 100 mg of sample with 2.5 N HCl for 3 h; hydrolyzate was neutralized with sodium carbonate and filtered with Whatman, and total soluble sugar released was determined. Aqueous extraction of reducing sugar from samples was performed according to that of soluble sugar and reducing sugar, but the supernatant was determined by Miller method using dinitrosalicylic acid reagent (DNS) (Miller 1959).

Determination of enzyme assays

The α -amylase enzyme activity was measured as previously described using 0.2% soluble starch (Sigma) dissolved in

boiling 0.05 M KH_2PO_4 –NaOH buffer (pH 6.0), and cooled to 40 °C. To each sample tube, 1 ml crude enzyme solution was mixed with 1 ml buffered starch solution (substrate) allowed to react for 10 min at 40 °C in water bath before adding 1 ml fresh iodine reagent (0.5% I_2 in 5.0% KI) (Saheed et al. 2013b). Absorbance of triplicate sample was measured at 620 nm; one unit of α -amylase is defined as the amount of enzyme that hydrolyze 0.1 mg of starch in 10 min at 40 °C when 4.0 mg of starch is present. Cellulase enzyme activity assay was conducted as outlined by the NREL LAP-006 (Adney and Nrel 2008) with modifications. All triplicate crude enzyme extracts were added to 50 mg substrate (1.0 × 6.0 cm Whatman no. 1 filter paper strip) was mixed with 0.05 M Na-citrate buffer at pH 4.8 to make 1.0 ml final volume and incubated at 50 °C for 60 min. One unit enzyme activity was defined as the amount of enzyme that produced 1 μmol of sugar equivalent as glucose calculated using glucose standard curve. Amount of glucose release was measured using the phenol–sulphuric acid assay (Dubois et al. 1956).

Fungal chitin measurement

Fungal chitin (glucosamine) concentration was analyzed based on previously described method (Chen and Johnson 1983). Twenty milligrams of dried sample were refluxed at 100 °C for 4 h in 10 ml of 6 N hydrochloric acid. Hydrolysate was filtered, and 1 ml aliquot was evaporated to dryness at 60 °C and re-dissolved in 4 ml distilled water. 0.25 ml of acetylacetone (4% acetylacetone in 2.5 M sodium carbonate) in 1 ml of solution was heated at 90 °C for 1 h, and after cooling, 2 ml of ethanol and 0.25 ml of Ehrlich reagent were added before absorbance was measured at 530 nm. The glucosamine standard curve was used to measure the fungal chitin.

Results and discussion

Growth kinetics of *P. chrysosporium* and *S. commune* mixed culture

In the conventional batch SSF involving monoculture fungi, the growth pattern usually involves brief lag phase, exponential, and a death phase (Mishra et al. 2017; Gupta and Jana 2018). It is interesting to report that the growth pattern of *P. chrysosporium* and *S. commune* mixed culture in the present investigation showed similar pattern and the exponential growth phase lasted until day 5 before decline. This shows that, regardless of culture composition, a batch process would maintain the conventional growth phases (Mishra et al. 2017). Therefore, a plot of " $\ln \mu$ " against fermentation period depicted first-order kinetic model as $\ln \mu$ increased as the fermentation

progressed to day 5 (Fig. 2a) with R^2 of 0.951 and specific growth rate of 0.498 day^{-1} . Microbial growth in batch SSF is characterized with the early filamentous penetration of substrate bed due to availability of nutrients, oxygen, and favorable growing conditions (Matsakas et al. 2014). Similarly, at death phase (day 6–9), the death pattern was linear (Fig. 2b) with R^2 of 0.829 and -0.190 day^{-1} as specific growth rate at death phase. The reduction in both R^2 value and growth rate after 6 days showed consistency of mixed fungi cultivation with classical monoculture batch systems and this suggested that metabolic characteristics of novel mixed white rot fungal culture could be process-specific rather than interaction specific as previously reported (Song et al. 2010; Shi et al. 2012).

The classical Monod model (non-inhibitory) and Haldane (inhibitory) type of the models (Surendhiran et al. 2014) that relate microbial growth rate with substrate consumption were, for the first time, applied to test the fitness of experimental data of mixed culture of *P. chrysosporium* and *S. commune* to the models. A positive correlation will show congruency of the model regardless of number or type microbial class cultivated. Thus, the maximum microbial growth rate ($\mu_{\max}$), substrate constant (K_s), and substrate inhibition constant (K_i) of the batch process were determined and compared.

Data obtained showed that classical Monod and modified model described by Haldane adequately fitted the process, but the latter describes the process better (Fig. 2c, d). Though the value of substrate constant was generally tolerable (Mullai et al. 2013), Haldane model further showed slight substrate inhibition at high concentration (Table 1). The result suggested a positive relationship between monoculture and mixed culture SSF in a batch process as values recorded corroborate observation of other monoculture investigations where elevated substrate concentration caused inhibition of microbial growth. Hence, it seems that metabolism of mixed culture batch SSF systems is consistent with monoculture SSF in terms of overall microbial growth and substrate consumption pattern (Ravi et al. 2013). The maximum growth rate accounted for by the models is comparable (Fig. 2c, d) albeit, Haldane model recorded slightly higher values and this could be because the model accounted for inhibition caused by elevated substrate in the SSF process (Song et al. 2010; Surendhiran et al. 2014). The increased microbial growth could be result of higher concentration of available substrate sugar or the synergism offered by the fungal mixed culture as previously reported (Saheed et al. 2013a; Jamal et al. 2014).

Kinetics of product formation

Protein synthesis and substrate consumption

One of the main products in this SSF process whose production kinetics remained ignored by investigators is protein

Fig. 2 Growth of the fungal mixed culture; **a** growth during exponential growth phase; **b** growth during death phase; **c** observed data of specific growth rate of fungal mixed culture and model fit of non-inhibitory Monod growth kinetic model; **d** observed data of specific growth rate of fungal mixed culture and model fit of Andrews' inhibitory kinetic model

Table 1 Values of kinetic parameters associated with classical Monod model and its modification

Kinetic model	Kinetic parameters			
	K_s (mg/g)	K_i (mg/g)	$\mu_{\max}$ (day ⁻¹)	R -squared (R^2)
Non-inhibitory Monod model	23.35	NA	0.641	0.908
Inhibitory Monod model (Haldane)	0.40	233.37	0.644	0.932

NA not applicable

Fig. 3 Luedeking–Piret model of the fermentation process: **a** product formation kinetics; **b** substrate consumption

synthesis by the mixed fungal culture. The mathematical equation described by Luedekin–Piret (Manikandan et al. 2008; Mullai et al. 2013) that involves a plot of specific growth rate (μ) versus product formation produced a straight line whose slope is the growth-associated co-efficient (α) and the intercept is non-growth-associated parameter (β) was used to observe protein synthesis by fungal mixed culture. The model adequately described the product synthesis having R^2 of 0.988 (Fig. 3a). Similarly, the values of the model co-efficient(s) showed that product formation is not fungal growth-dependent (Table 2). The non-growth association could be attributed to the presence of other extracellular products such as cellulase and α -amylase enzymes that are equally proteins, but could only be responsible for the conversion of complex carbohydrates in the substrate to simple sugars (Raghavarao et al. 2003; Sethi et al. 2016). Result reported here showed that product formation determination after microbial growth could be product location

specific (intracellular or extracellular). Bio-hydrogen under SSF and biodiesel production were both growth-dependent in the presence of different substrates (Mullai et al. 2013; Surendhiran et al. 2014).

The low growth association recorded in the present study compared with those of monoculture kinetic study suggested that protein synthesis could be non-growth associated at elevated fungal growth (Arumugam and Manikandan 2011). Substrate consumption generally decreased as fermentation period increased suggesting a direct relationship and consistency with Luedeking–Piret kinetic equation as applicable to product formation (Mullai et al. 2013). The slope of the graph (-49.08 mg/g) (Fig. 3b) represents the growth-associated substrate consumption co-efficient (γ) and the intercept (48.86 mg/g) is non-growth-associated substrate parameter (λ). The R^2 value of 0.938 showed adequate fit of the model to experimental values, while the values of γ and λ depicted active substrate degradation during product formation. Kinetics of ethanol production by *Saccharomyces cerevisiae* was growth associated under optimum substrate consumption (Manikandan et al. 2008). Though still difficult to obtain comparable data of mixed fungal culture production kinetics, mangrove sediment microorganism offers growth-associated bio-hydrogen production with comparable kinetics description (Nath and Das 2011).

Kinetics of enzyme synthesis

Cellulase and α -amylase enzyme synthesis of the fungal mixed culture differ from their monoculture counterparts as increased synthesis was recorded over the mixed culture media. The average cellulase production by mixed culture of *P. chrysosporium* and *S. commune* reached 40 IU/gds within 6 days, while monoculture of individual strain cultivated for 20 days gave 15.2 and 23.1 (IU/gds) under SSF (Govumoni et al. 2015; Zhu et al. 2016). The mixed fungal culture also gave higher amylase (395.6 IU/gds) compared with average of 23 IU/gds reported for monocultures cultivated under similar conditions (Onofre et al. 2012). Production of extracellular enzymes by white rot fungi under SSF could depend on fermentation period (Pandit and Maheshwari 2012; Melikoglu et al. 2013). Although enzyme synthesis by the mixed fungal culture was higher, synthesis could be synergistic rather than individualistic (Juhász et al. 2003).

Table 2 Numerical values of kinetic parameters associated with product synthesis

	Protein synthesis kinetics			
	γ (mg/g)	λ (mg/g)	α (mg/g)	β (mg/g)
Monoculture (Manikandan et al. 2008; Surendhiran et al. 2014)	0.232	0.279	0.125	0.002
Mixed culture (this work)	49.080	48.860	0.015	0.052
Hanes–Woolf	Enzymatic activity kinetics			
	$V_{\max}$ (IU/gds/day)	K_m (IU/gds)	R^2	
α -Amylase	25.19	11.55	0.911	
Cellulase	3.05	57.47	0.988	

 α : growth-associated product synthesis co-efficient β : non-growth-associated product synthesis co-efficient γ : growth-associated substrate consumption co-efficient λ : non-growth-associated substrate consumption co-efficient

NA not applicable

Fig. 4 Hanes–Woolf model of enzyme kinetics; **a** α -amylase production during bioconversion process; **b** cellulase production during bioconversion process

Kinetic constants of α -amylase and cellulase enzymes activity were determined using Hanes–Woolf equation. The model adequately describes the enzymes activity with R^2 of α -amylase and cellulase enzymes exceeding 0.9 (Fig. 4a, b). The α -amylase enzyme maximum velocity ($V_{\max}$) and rate constant (K_m) were 25.19 IU/gds/day and 11.55 IU/gds/day. The positive K_m value obtained showed profound activity by α -amylase in the biochemical process. Activity of extracellular hydrolytic enzymes has been suggested to influence biochemical processes even at low rate constants (Ghori et al. 2012; Mfombep et al. 2013). Enzymatic activity recorded in this investigation is comparable to the other reports involving fungal monocultures in terms of production and kinetics (Melikoglu et al. 2013; Sethi et al. 2016). The values of Hanes–Woolf kinetic model for cellulase enzyme (Fig. 5b) revealed positive influence on the biochemical process with $V_{\max}$ of 3.05 IU/gds/day and K_m of 57.47 IU/gds/day. The high K_m value for cellulase enzyme at low titer could mean that the enzyme is specific in producing simple sugars from substrate cellulose to support mixed fungal growth. Production of low cellulase enzyme titer has been reported for fungal monoculture (Alam et al. 2005); observing such low titer under fungal mixed culture suggests that enzyme activity could not have been microbial system-dependent.

Fig. 5 Effects of substrate depth in tray reactor on products synthesis; **a** protein and biomass production; **b** enzyme cellulase and α -amylase enzyme activity

Fermentation process in tray reactor

Tray reactor is low cost and requires limited space (Singh *et al.* 2009; Nigam and Pandey 2009; Arora and Sharma 2011). The kinetic outcomes in the shake flask were considered adequate for measuring the performance of the tray reactor via yield and productivity comparative to flask outcomes. Effects of substrate depth and bioconversion period influence on SSF were also determined.

Tray reactor productivity and yield analysis

The μ and all yield parameters showed higher values in the reactor compared with shake flask (Table 3). High yield is characteristic of SSF, since it mimics fungal natural-growing conditions (Lizardi-Jiménez and Hernández-Martínez 2017). Similar result has been reported for improved yield in tray reactor (Arora and Sharma 2011; Dhillon *et al.* 2011). However, the amount of fungal biomass recorded was in tray reactor. This condition could be responsible for non-growth association recorded in the shake flask SSF kinetics.

The productivity of reactor in this investigation is more than the shake flask productivity despite increased fungal biomass in the latter. The kind of material used in designing reactors and overall growth consistency of fungi cells in SSF have been reported to affect reaction process, moisture

Table 3 Yield and productivity in shake flask and reactor

Parameter	Shake flask	Reactor
$Y_{p/x}$ (w/w)	3.77	40.88
$Y_{p/s}$ (w/w)	0.64	0.66
$Y_{x/s}$ (w/w)	0.02	0.02
$Y_{x/p}$ (w/w)	0.04	0.02
μ (mg/g)	0.47	0.49
Productivity (mg/g/day)	28.15	32.65

$Y_{p/x}$: product yield in relation with biomass

$Y_{p/s}$: product yield in relation with substrate

$Y_{x/s}$: biomass production in relation with substrate

μ : specific growth rate

loss, temperature build up, and product quality (Chelliappan 2013; Zhang *et al.* 2013). Therefore, reactor material was suspected to affect the microbial performance (Arora and Sharma 2011; Dhillon *et al.* 2011).

Influence of substrate depth on product formation in the reactor

The depth of the substrates in the reactor varied between 1.5 and 4.0 cm and the protein synthesis by the fungal mixed culture increased until 2.5 cm substrate depth; further increase resulted in low protein production (Fig. 6a). Low energy transfers and reduced hyphae penetration beyond 1.8 cm have been reported to reduce products formation by Oyster Mushroom and white rot fungi (Pandit and Maheshwari 2012). However, biomass concentration decreased throughout the fermentation course. The reduced oxygen availability in void spaces in the substrate depth was suspected to be responsible for less biomass production (Song *et al.* 2010; Mahanama *et al.* 2011; Jamal *et al.* 2015).

Cellulase and α -amylase enzyme synthesis with respect to substrate depth showed increased enzymatic activity as substrate depth increases, though synthesis plummets at depth more than 3.0 cm. Cellulase enzyme production fluctuates greatly as substrate depth increased to 3.5 cm where the highest synthesis was recorded (Fig. 4b). Fungal hyphae penetration through the substrate thickness could be responsible for elevated enzymatic activities. The fluctuations in cellulase synthesis could, however, be process-specific (Alam *et al.* 2005); product synthesis variation is characteristic of SSF under incremental substrate thickness (Mahanama *et al.* 2011; Pandit and Maheshwari 2012).

Effects of fermentation period on product formation in tray reactor

The influence of fermentation time on products synthesis in the reactor was observed (same in shake flask), and the results

Fig. 6 Effects of fermentation period in tray reactor on product synthesis: **a** protein synthesis and biomass production; **b** α -amylase and cellulase enzymes activities

obtained showed that protein synthesis is growth-dependent, since the gradual increase in fungal biomass led to increased product formation from day 2 to day 6 (Fig. 5a). The gradual increase in product synthesis could be due to increased cellular activity in the SSF process. This trend is often reported for batch protein production by fungal systems (Jamal et al. 2012; Alemu 2013). However, the short period exhibited in reaching optimum product formation suggested synergism by the mixed fungal culture (Ruqayyah et al. 2011). The decline in protein synthesis after day 6 could be caused by fungal growth inhibition due to products synthesis, substrate, and oxygen depletion (Ruqayyah et al. 2013).

Enzymatic (cellulase and α -amylase enzyme) activities followed the protein synthesis pattern, but the highest synthesis was at day 4 (Fig. 6b). The outcome suggested that cellulase and α -amylase activities could not be culture-dependent (Pandit and Maheshwari 2012; Lizardi-Jiménez and Hernández-Martínez 2017).

Conclusion

The results obtained in this study showed that Haldane and Monod kinetic models were able to describe the kinetics of combined biomass growth of fungal mixed culture, while

Leudeking–Piret model sufficiently described product formation kinetics. In addition, the kinetic models showed that the product formation is less growth associated, while substrate consumption is growth associated. Finally, it was evidenced that the mixed fungi cultivation in the tray reactor compared adequately with shake flask and product synthesis depends on substrate thickness. Using the fruit peels as animal feed after protein enrichment by mixed culture of *P. chrysosporium* and *S. commune* offers economic value than landfilling.

Acknowledgements The investigators would like to thank Research Management Center of International Islamic University Malaysia for their financial support in making this work a reality.

Compliance with ethical standards

Conflict of interest There are no financial/commercial or any other conflicts of interest among authors.

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