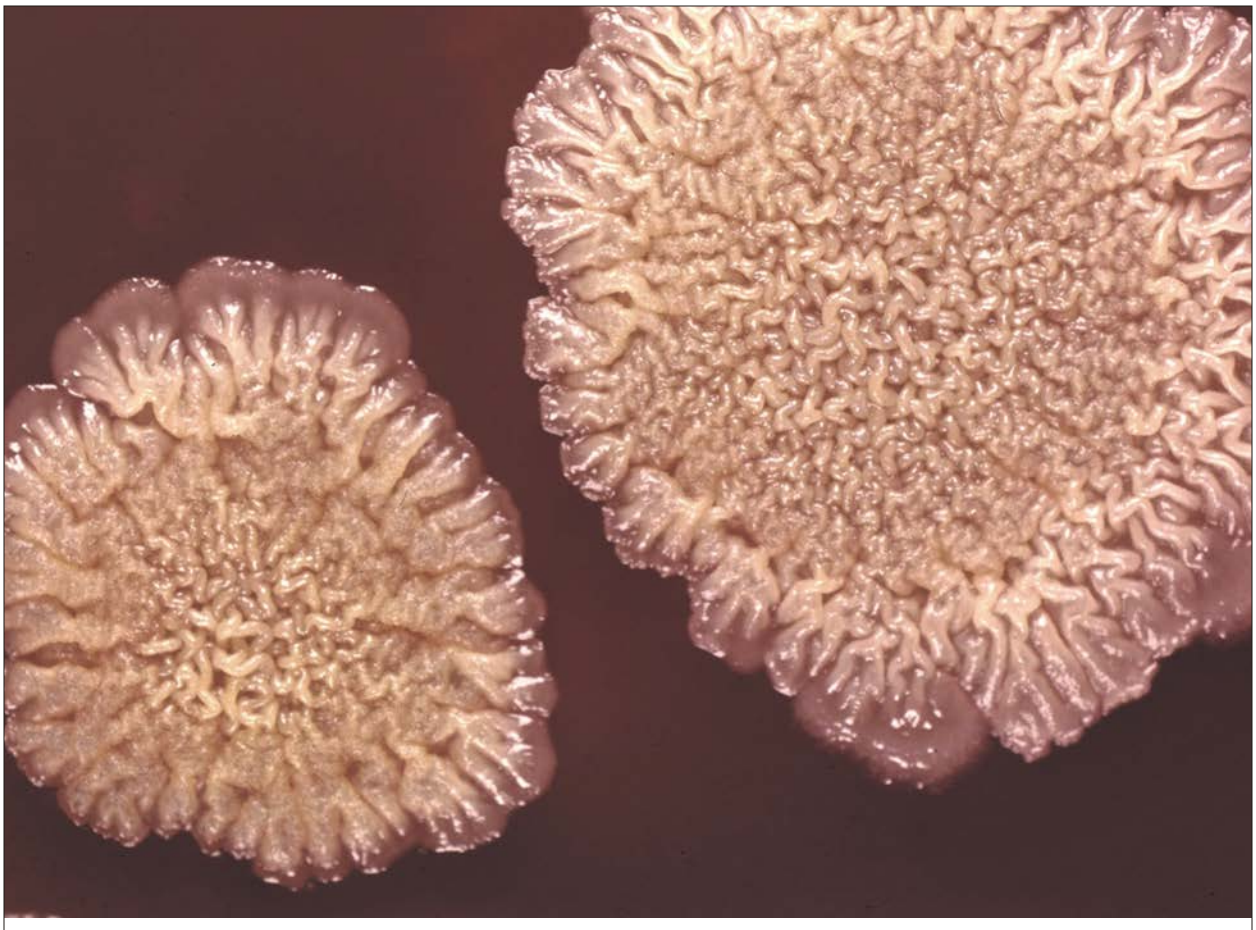

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Effects of Inoculum and Substrate Concentrations in Anaerobic Fermentation of Treated Rice Bran to Acetone, Butanol and Ethanol

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ABSTRACT

The availability of ricebran; one of the by products of the rice-milling industry in cheap and large quantity, is utilised in this work for the production of renewable fuel through fermentation process. Treated ricebran, a lignocellulosic material considered as wastes from the rice milling industry was fermented anaerobically in a batch process using *Clostridium saccharoperbutylacetonicum* N1-4 as the inoculum at initial pH of 6±0.2 and temperature of 30°C for the determination of the most suitable initial concentration of ricebran and the inoculum concentration that will produce the maximum amount of products. Different ricebran initial concentrations, 10, 20, 50, 100 and 120 g L^{-1} were fermented using inoculum concentration of 1.5 g L^{-1}. Effect of Inoculum concentration was also investigated by using inoculum concentrations of 0.5, 1.0, 1.5, 2.0 and 2.5 g L^{-1} for fermentation of ricebran hydrolysates at fixed substrate concentration and volume. Increment in substrate concentration had a significantly positive effect ($p<0.05$) on yield and productivity of acetone, butanol and ethanol up till concentration of 100 g L^{-1} after which further increment led to reduction in the yield and productivity. In addition, increment in inoculum concentration also had a significantly positive effect ($p<0.05$) on yield and productivity of the fermentation products up till concentration of 1.5 g L^{-1} after which further increment in inoculum concentration led to decrease in the yield and productivity. The highest products'(acetone, butanol and ethanol) yield and productivity; 0.39 g g^{-1} and 0.079 g L^{-1} h^{-1}, respectively, were obtained at the initial ricebran concentration of 100 g L^{-1}. In addition, the highest products' (acetone, butanol and ethanol) yield and productivity; 0.39 g g^{-1} and 0.095 g L^{-1} h^{-1}, respectively were obtained at an inoculum concentration of 1.5 g L^{-1}. Higher inoculum concentration resulted in lower yield and productivity of acetone, butanol and ethanol. The best inoculum concentration and substrate concentration for production of acetone, butanol and ethanol from treated ricebran are 1.5 and 100 g L^{-1}, respectively.

Key words: Rice bran, acetone/butanol/ethanol fermentation, initial substrate concentration, inoculum concentration, *Clostridium saccharoperbutylacetonicum* N1-4

INTRODUCTION

The growing demand for biofuel as an alternative to fossil fuel is occasioned by the adverse effects associated with the continuous usage of fossil fuel. Some of these effects which include

greenhouse gas effects, limiting resources and high cost of production have led to an intense search for strategic planning mechanism. This mechanism is expected to ensure a successful commercial production process for biofuel as well as sustenance within the system. The special attributes of biofuel which include non-toxicity, biodegradability, renewability and cheaper cost of production have indicated that biofuel holds the future of energy generation (Khamaiseh *et al*., 2012).

The prospects of microbial fermentation through the use of *Clostridium* sp. to assume a vantage position as a foremost biofuel generating technique is being hindered by problems such as low yield and unavailability of raw materials. However, intense efforts by researchers have led to an explosion of research articles which has translated into new concepts on how to combat this setback and increase the yield and productivity of products. Some of these new concepts have resulted in the prediction of large increment in the yield and productivity of products in such a way that productivity with actual substrates under feasible conditions is moving towards a more achievable value (Lee *et al*., 2008). A major factor in the production of acetone, butanol and ethanol (ABE) is the availability and affordability of the substrate. Productions of ABE from various raw materials and renewable agricultural materials have been reported (Qureshi *et al*., 2008; Ezeji and Blaschek, 2010; Khamaiseh *et al*., 2012).

Ricebran (RB) is a lignocellulosic biomass which is obtained as one of the by-products in the rice-milling industry. The world's annual paddy rice production is more than 500 million metric tonnes and out of this enormous amount, RB, one of its primary wastes product accounts for about 40 million metric tonnes. RB comprises of about 15-20% oil, 12-16% protein, 6.5% oligosaccharides, 35-55% other carbohydrates and 7-10% silica and other micro elements (Wang *et al*., 2005). RB is considered as not suitable for human consumption but used largely as supplements for animal feeds due to its high fiber content, possible hull contamination and susceptibility to rancidity if kept for a long time (Saunders, 1985).

Pretreatment is a major factor that influences the yield and productivity of fermentation products as it determines the amount of simple sugar released for fermentation process (Lee *et al*., 2009). Pretreatment may be via acid hydrolysis or enzymatic hydrolysis or by both.

Acid hydrolysis (pretreatment) is a process used to degrade macromolecular materials such as lignocellulose into simpler compounds. This phenomenon has found high utility in the conversion of cellulose and other polysaccharides into reducing sugars that can be easily fermented by microorganisms (Panagiotopoulos *et al*., 2009). Enzymatic hydrolysis refers to the treatment of the biomass with enzyme, this process is to compliment the acid hydrolysis in order to breakdown the complex lignocellulosic structure into simple sugar fermentation process.

Initial Substrate Concentration (ISC) and initial inoculum concentration are two other important factors that greatly influence the yield and rate of productivity of fermentation products (solvents and acids). Although, recent research studies have focused on utilization of substrate as this is recognized as a major factor to be considered if substrate utilization is to be efficiently managed for optimum biofuel production. Dong *et al*. (2012) stated that extremely low substrate concentration limits the rate of ABE formation while very high concentration may inhibit product formation. In the same vein, low inoculum concentration adversely affects the mechanism of fermentation process thus causing a much lower rate and yield of ABE production, whereas, very high cell concentration causes increased activities of organisms which end up utilizing the fermentation end results for their growth and in the process limit the yield and rate of formation of fermentation products as well (Lee *et al*., 2008).

Based on the above observations, the objective of this study was to determine the effects of initial RB hydrolysates and inoculum concentration on the production of Total Volatile Fatty Acid

(TVFA), yield and productivity of ABE in a batch anaerobic fermentation process using *C. saccharoperbutylacetonicum* N1-4 as the inoculum. This is with a view to knowing the appropriate initial substrate and inoculum concentrations that can ensure the best ABE production. In addition, the suitability of Trichloroacetic Acid (TCA), an organic acid, as a probable pretreatment agent for biomass was also investigated.

MATERIALS AND METHODS

Year of study: The various experiments in this study as described below were conducted between December 2010 and December 2011 at Biotechnology Pilot Test Laboratory of Department of Chemical and Process Engineering, Universiti Kebangsaan Malaysia.

Feedstock: Freshly milled RB sample was obtained from Kilang Beras BERNAS, Tanjung Karang, a local rice-milling company in Selangor, Malaysia. 10-mesh particle size sieve was used to sieve the sample to ensure a uniformly sized sample. The RB was preserved dry and away from light in a dark air tight dry container and stored in a cold room for further use. The temperature of the cold room was maintained between 8 and 12°C. Oil containing materials under storage at room temperature undergo rancidity when exposed to light over a long period of time (Allen and Hamilton, 1989). The use of RB as substrate was considered for this study because it contains a mixture of carbohydrates, in addition to this, it is readily available at cheap cost as well as largely unsuitable for human consumption. Furthermore, the usage of RB will assist in conversion of wastes to useful products with little or no contribution to the net environmental pollution.

Design of experiment: One-factor design method, otherwise called one variable at one time (OVAT)-one of the simplest tools in design of experiment was adopted in this study. This method was used to screen the factors (input variables being investigated i.e. concentrations of substrates and inoculum). After the screening, 10, 20, 50, 100 and 120 g L^{-1} were adopted as levels for one of the factor (substrate concentration) while 0.5, 1, 1.5, 2 and 2.5 g L^{-1} were adopted as the levels for inoculum concentration-the other factor to be investigated. 'One factor at five levels' mode of experiment was then carried out, under this mode, only one factor out of the two being investigated was varied while the other was kept constant. In this study, two blocks of experiments were adopted. In the first block, hydrolysates with different values of substrate concentration (stated above) were inoculated with inoculum of 1.5 g L^{-1} inoculum concentration while in the second block of experiment, different inocula concentrations (stated above) were used to inoculate hydrolysates of 100 g L^{-1} substrate concentration. Three replications of each experiment were performed. This method (OVAT) was adopted because of its simplicity of operation and ease of interpretation (Meilgaard *et al*., 1999).

Acid hydrolysis and enzymatic hydrolysis: Sample of RB (10 g) was suspended in 1 L distilled water acidified with 1% (w/v) of TCA to generate RB hydrolysate. Control experiment for RB was also set up using distilled water only. The mixture was left for 1 h at room temperature. Thereafter, the pH of the acid treated sample was adjusted to pH 4.8 with 10 M NaOH, this was done in preparation for the enzymatic process that was to follow. The enzymes used in this study were cellulase (Celluclast 1.5 L, Sigma Chemicals) and β-glucosidase (Novozyme 188, Sigma Chemicals). Results of previous studies where these enzymes have been used for hydrolysis showed that the range of pH under which these enzymes are most active was between pH 4.5 and pH 5

(Ghose, 1987). The sample was then subjected to simultaneous enzymatic hydrolysis with a cellulase (Celluclast 1.5 L, Sigma Chemicals) loading of 6 mL 100 g^{-1} sample and β-glucosidase (Novozyme 188, Sigma Chemicals) loading of 6 mL 100 g^{-1} sample in an incubating orbit shaker for 72 h at 45°C, the speed of rotation was 170 rpm (Lee *et al.*, 2009). After enzymatic hydrolysis, the pH of the treated samples was then adjusted to pH 7 with 10 M NaOH and samples were taken for total sugar analysis. The samples were then used as hydrolysates for fermentation process. The above procedures were repeated mixing 20, 50, 100 and 120 g each with 1 L distilled water to generate 20, 50, 100 and 120 g L^{-1} concentrations of treated RB samples.

Microorganism and inoculum preparation: *C. saccharoperbutylacetonicum* N1-4 which was used in all experiments in this work was obtained from the stock culture of the microorganism managed in the Biotechnology Pilot Plant Laboratory of the Department of Chemical and Process Engineering of UKM. It was maintained as a suspension of spores in a potato glucose medium (PG medium) as a stock culture and kept at 4°C. In preparing the inoculum, the spores in suspension were transferred into a Potato Glucose medium at ratio 1:10 mL. The mixture was subjected to heat shock treatment. This involved heating the mixture in boiling water for 1 min after which it was then dipped into a container of ice water for immediate cooling. The mixture was then incubated at 30°C for 48 h under anaerobic conditions. The fresh culture was then transferred into a freshly prepared sterilized Tryptone-yeast extract-acetate medium (TYA medium) and was incubated at 30°C. The growth of the cell-biomass was monitored and determined using Hach DR2800 spectrophotometer (Hach, Colorado, USA) at 660 nm described elsewhere (Sutton, 2006).

Composition of inoculum and fermentation media: PG medium: This medium consisted of 150 g potato, 10 g glucose, 0.5 g $(NH_4)_2SO_4$ and 3 g $CaCO_3$ in 1 L of distilled water.

TYA medium: This medium consisted of 20 g glucose, 6 g tryptone, 2 g yeast extract, 3 g CH_3COONH_4, 0.5 g KH_2PO_4, 0.3 g $MgSO_4.7H_2O$ and 0.01 g $FeSO_4.7H_2O$ in 1 L of distilled water.

Fermentation medium: 6 g Tryptone, 2 g Yeast extract, 3 g CH_3COONH_4, 0.5 g KH_2PO_4, 0.3 g $MgSO_4.7H_2O$ and 0.01 g $FeSO_4.7H_2O$ in 1 L of distilled water.

Batch fermentation process: All fermentation experiments were conducted using 250 mL Duran bottle with the working volume of 100 mL. The treated hydrolysates were used as the sole source of carbon in the fermentation medium. The pH of the medium was adjusted to 6.0±0.2 after which the medium was then sterilized by autoclaving at 121°C, 15 psi for 15 min. After the sterilization, an anaerobic condition was attained by passing nitrogen gas through the medium for about 1-2 min. in a sterilized environment and the medium was thereafter inoculated with the freshly prepared *C. saccharoperbutylacetonicum* N-14 as described above. The volume of inoculum was 10% of the fermentation medium. The incubator was maintained at 30°C and subjected to agitation at 120 rpm. This was to bring about a complete homogeneity in the fermentation chamber. Samples were taken at designated intervals for analysis. All the experiments were conducted in triplicates and the average of the values were reported.

Analytical methods: Samples were taken at designated intervals. The samples were centrifuged at 7,500 xg for 10 min and the supernatant were filtered using 0.2 μm cellulose acetate filter.

Sugar analysis: Composition of fermentable sugars prior to and during fermentation and their concentration were determined using high-performance liquid chromatography (HPLC Agilent 12000 series, Agilent technologies, Palo Alto, CA, USA). The HPLC is equipped with a Refractive Index Detector (RID) and a 300×7.80 mm RezexRCM-Monosaccharide Ca^{+2} (8%) column. The mobile phase which was 100% water was run at a flow rate of 0.6 mL min^{-1}. The temperature of the column was maintained at 70°C. The total run time was 18 min for each sample.

Solvent and acid analysis: Concentration of solvents (ABE) and acids were determined using Gas Chromatography (7890A GC-System, Agilent Technologies, Palo Alto, CA, USA) equipped with a flame ionisation detector and a 30-m capillary column (Equity1; 30 m×0.32 mm×1.0 μm film thickness; Supelco Co, Bellefonate, PA, USA). The oven temperature was programmed to increase from 40 to 130°C at a rate of 8°C min^{-1}. The injector and detector temperatures were set at 250 and 280°C, respectively. Helium, as the carrier gas, was set at a flow rate of 1.5 mL min^{-1}.

Inoculum concentration was determined using a Hach DR2800 spectrophotometer (Hach, Colorado, USA) at 660 nm. The ABE productivity was defined as the ABE concentration divided by the total fermentation time given by g L^{-1} h while the ABE yield is defined as the total ABE divided by the total carbohydrate consumed and given by g g^{-1} (Jesse *et al*., 2002).

Statistical analysis: The data obtained from the experiments above were subjected to statistical analysis using one-way analysis of variance (ANOVA) in Statistical Package for Social Sciences (SPSS) analytical tool. One-way ANOVA was adopted in this study to analyse and compare the variability of the values of concentrations of inoculum and substrate used in the experiments and to determine if these values (concentrations) have significant effects on yields and productivities of the fermentation end products. In addition, this method was adopted because only one independent variable (either substrate concentration or inoculum concentration) was investigated at a time. Tukey's Post Hoc tests were also carried out to know the extent of interaction between the different levels of factor under consideration. The statistical tests were carried out in order to know if there is a statistically significant difference in the data recorded since it is necessary to determine if real difference; as defined by statistics, exists between the means of the values.

RESULTS AND DISCUSSION

Sugar analysis and consumption in RB hydrolysates: Table 1 shows the composition and consumption of the different sugars present in the different RB hydrolysates used to investigate the effect of the Initial Substrate Concentration (ISC). The amount of total sugar in the hydrolysates was directly proportional to the ISC, the smallest (8.80 g L^{-1}) was obtained from the hydrolysate with the least ISC (10 g L^{-1}) while the highest total sugar (34 g L^{-1}) was obtained from the hydrolysate with the highest ISC (120 g L^{-1}). There was an increment of 60.9% (more than half of initial value) in the total sugar content when the ISC was doubled initially from 10 to 20 g L^{-1}. This increment was consistent, as an increment of almost the same magnitude (50.1%) was observed when ISC was increased from 50 to 100 g L^{-1}. The amount of sugar obtained, which was as a result of the effect of ISC, eventually affected the products of fermentation process as will be seen shortly. Glucose was observed as the dominant sugar in the hydrolysates and it was observed that of all the different sugars that were present in the hydrolysates, the hexoses (glucose and fructose) were mostly consumed with glucose being almost totally consumed while sucrose and xylose were hardly consumed. This showed that the microorganism

Table 1: Effect of different substrate and inoculum concentrations on sugar composition and utilisation

	Conc. (g L^{-1})								
	Glucose		Fructose		Sucrose		Xylose		Total sugar
Conc. (g L^{-1})	Initial	Final	Initial	Final	Initial	Final	Initial	Final	consumed
Substrate									
10	8.696771	0.025627	0.024380	0.021188	0.029239	0.029239	0.051914	0.051914	8.674336
20	13.407880	2.561618	0.676523	0.009889	0.029239	0.029239	0.051914	0.051914	11.512900
50	11.765790	0.025627	0.024380	0.024380	7.354348	0.029239	0.051914	0.051914	19.065270
100	28.538710	4.228901	0.024380	0.024380	0.029239	0.029239	0.051914	0.051914	24.309810
120	32.451350	10.467210	1.323667	0.024380	0.029239	0.029239	0.688833	0.051914	23.920350
Iuoculum									
0.5	31.95480	20.771000	0.024376	0.024376	0.029239	0.029239	0.051914	0.051914	11.18381
1	29.03989	5.488291	0.024376	0.024376	0.029239	0.029239	0.051914	0.051914	23.55160
1.5	29.03989	0.025627	0.024376	0.024376	0.029239	0.029239	0.051914	0.051914	29.01426
2	31.95480	8.420573	0.024376	0.024376	0.029239	0.029239	0.051914	0.051419	23.53472
2.5	28.60000	10.786060	0.024376	0.024376	0.029239	0.029239	0.051914	0.051914	17.81394

(*C. saccharoperbutylacetonicum*) is diauxic in nature and this observation is consistent with an earlier observation by Ounine *et al.* (1985). Table 1 shows 100% increment in ISC from 10 to 20 g L^{-1} led to a reduction of 19% in glucose consumption (i.e., instead of doubling the amount of sugar consumed to 8.674336×2, the actual amount consumed (11.5129 g L^{-1}) was less by 19% of the expected amount) while the increment in ISC from 50 to 100 g L^{-1} resulted in 15% reduction in glucose consumption. This may partially be due to the increasing substrate/microorganism ratio considering the fact that a constant inoculum concentration was used while the ISC was varied. In addition, the reason for this may be ascribed to the inhibitory effects of substrates. This implies that for very high values of ISC, there will be significant reduction in the amount of glucose consumed because, the presence of more substrate is likely to inhibit the metabolic pathway of the sugar consumption. This may be why the yield and productivity of the hydrolysate whose ISC is 120 g L^{-1} were lower than those of hydrolysates of lower ISC. Although, in agreement with observation by previous researchers, extremely low ISC (10 g L^{-1}) also resulted in low yield and productivity of ABE (Dong *et al.*, 2012), however, this may possibly be due to substrate limitation.

Variations of initial inoculum concentration also had effects on the pattern of sugar consumption. From the investigations carried out, it was observed that glucose was the mostly consumed sugar while other sugars such as fructose, xylose and sucrose were barely consumed (Table 1). It was also observed that glucose consumption increased as the inoculum concentration increased from 0.5 to 1.5 g L^{-1} and further increment beyond this value led to reduction in sugar consumption. For instance, despite having the same ISC, 34% of the available glucose was consumed in the hydrolysate inoculated with an initial inoculum concentration of 0.5 g L^{-1} while 99% of the available glucose in the hydrolysate inoculated with initial inoculum concentration of 1.5 g L^{-1} was consumed. These observations agreed with the observations of Lee *et al.* (2009) from an earlier study. The low amount of sugar consumed which is observed in hydrolysates inoculated with inoculum of low concentration may be due to limitations on the part of the cells by the extremely low cell/substrate ratio considering the fact that hydrolysates of same ISC was used in this investigation. However, the reduction in the consumption of glucose in hydrolysates inoculated with higher inoculum concentration could have been as a result of product inhibition.

Effect of ISC on TVFA and solvent productions: The amount of TVFA produced during fermentation as well as the yield and rate of production of ABE are greatly influenced by the initial substrate concentration. The profile of the TVFA produced during fermentation using different initial substrate concentrations is shown in Fig. 1a. At a fixed initial inoculum concentration of 1.5 g L^{-1}, the TVFA decreased as the initial substrate concentration increased up to 100 g L^{-1} i.e., 3.29, 2.63 and 2.63 g L^{-1} at 20, 50 and 100 g L^{-1} substrate concentration, respectively. Further increment in substrate concentration (120 g L^{-1}) led to increased TVFA production (6.04 g L^{-1}). Fig 1b shows the ABE profile of the fermentation process. The ABE increased with increasing ISC up till 100 g L^{-1}, i.e., 0.014, 2.07, 6.25 and 9.57 g L^{-1} for 10, 20, 50 and 100 g L^{-1} substrate concentration, respectively, further increment in ISC i.e., 120 g L^{-1} resulted in reduced ABE (6.47 g L^{-1}). The very low ABE and TVFA at low ISC (10 and 20 g L^{-1}) may be due to substrate limitation (Table 2); as the sugar content of the substrate is used up, the formation of fermentation

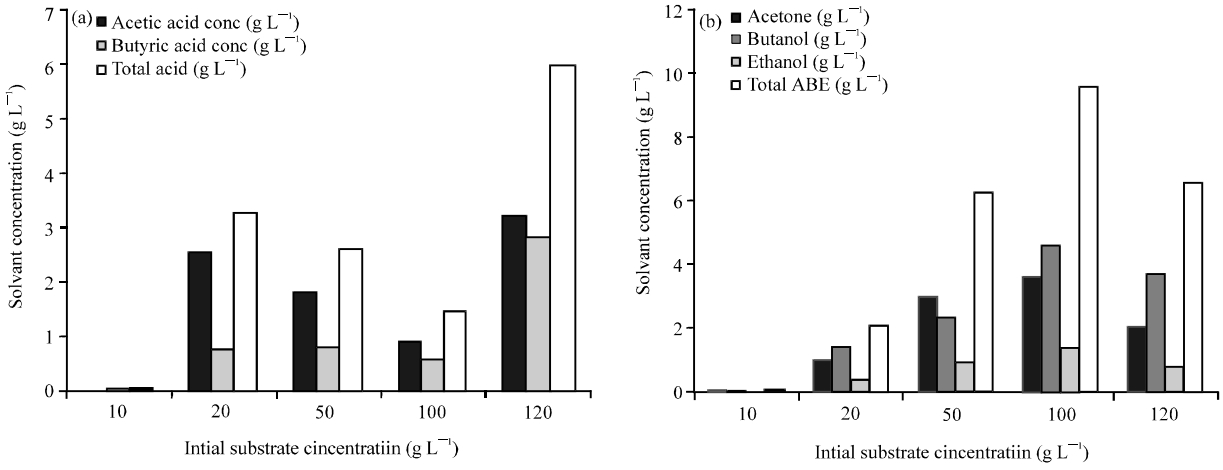

Fig. 1(a-b): (a) Total volatile fatty-acid and (b) ABE profile for initial substrate concentration, ABE: Acetone, butanol and ethanol

Table 2: Comparison of total ABE, yield and productivity at different substrate and inoculum coucentrations

	Conc. (g L^{-1})							
Couc. (g L^{-1})	Initial	Final	Consumed sugar (g L^{-1})	Total ABE (g L^{-1})	Yield (g g^{-1})	SD of yield	Productivity (g L^{-1} h^{-1})	SD of productivity
Substrate								
10	8.802304	0.127967	8.674336	0.014	0.001614	0.0001001	0.000117	0.0000537
20	14.165560	2.652660	11.512900	2.070	0.179798	0.0050060	0.017250	0.0024950
50	19.196430	0.131160	19.065270	6.250	0.327821	0.0159900	0.052080	0.0005424
100	28.644240	4.334434	24.309810	9.570	0.393668	0.0018570	0.079750	0.0001452
120	34.493090	10.572740	23.920350	6.470	0.270481	0.0665600	0.053910	0.0042080
Iuoculum								
0.5	32.06033	20.876530	11.18381	2.006	0.179366	0.0009558	0.016717	0.001138
1	29.14542	5.593820	23.55160	6.380	0.270895	0.0126600	0.053167	0.001712
1.5	29.14542	0.131156	29.01426	11.450	0.394633	0.0079010	0.095417	0.001723
2	32.06033	8.525607	23.53472	4.760	0.202254	0.0087760	0.039667	0.001341
2.5	28.70563	10.891590	17.81394	2.800	0.157180	0.0044320	0.023333	0.001625

ABE: Acetone, butanol and ethanol

Table 3: One-way ANOVA significance result for effect of substrate and inoculum concentration on yield and prodnctivity

	Sum of squares	df	Mean squares	F-value	Sig.
Substrate					
Yield					
Between groups	0.278	4.0	0.069	73.614	0.000
Within groups	0.009	10.0	0.001		
Total		0.287	14		
Prodnctivity					
Between groups	0.012	4.0	0.003	622.394	0.000
Within groups	0.000	10.0	0.000		
Total	0.012	14.0			
Iuoculum					
Yield					
Between groups	0.110	4.0	0.028	430.823	0.000
Within groups	0.001	10.0	0.000		
Total	0.111	14.0			
Prodnctivity					
Between groups	0.012	4.0	0.003	1257	0.000
Within groups	0.000	10.0	0.000		
Total	0.012	14.0			

products ceases. This is consistent with what has been observed by previous researchers (Argun *et al.*, 2008). Further increment in ISC beyond 100 g L^{-1} resulted in lower ABE yield (Table 2) probably because of inhibition by the substrate and the high TVFA value (Fig. 1a). The highest amount of total ABE (9.57 g L^{-1}), was obtained from the hydrolysate with ISC of 100 g L^{-1} (Fig. 1b). In addition, the highest yield and productivity, 0.39 g g^{-1} and 0.079 g L^{-1} h^{-1}, respectively were also obtained from the same hydrolysate (Table 2). It can also be seen that at higher ISC, there is accumulation of acids and this inhibits the fermentation process leading to a low yield of the products. High ABE yields had lower TVFA due to changes in the pathway of the metabolites which depends largely on the nature and hence the activities of the microorganism. It is speculated that higher initial substrate concentration above 100 g L^{-1} favored the activities of the organisms towards the production of more acids and less ABE. For all ISC, ABE yield increased with increase in ISC from 10 to 100 g L^{-1} where the highest yield of ABE was observed. After this value, ABE yield declined in value. This further confirms that higher ISC favor the production of acids, a process that inhibits the formation of ABE. The result of the one-way ANOVA statistical analysis of the data showed that substrate concentration had a significantly positive effect ($p<0.05$) on both yield and productivity of ABE (Table 3 extracted from the one-way ANOVA in SPSS statistical test). The standard deviations (Table 2) of the data for both yield and productivity showed that the average data set used did not deviate so much from the original values obtained in the actual experiments.

Effect of inoculum concentration on TVFA and solvent production: The profile of the TVFA produced during fermentation of different hydrolysates inoculated using inocula with different initial concentrations is shown in Fig. 2a while Fig. 2b shows the ABE profile of the fermentation process. At a fixed ISC of 100 g L^{-1}, the TVFA were 3.7, 3.65 and 1.32 g L^{-1}, respectively for initial inoculum concentration 0.5, 1 and 1.5 g L^{-1}. Further increment in inoculum

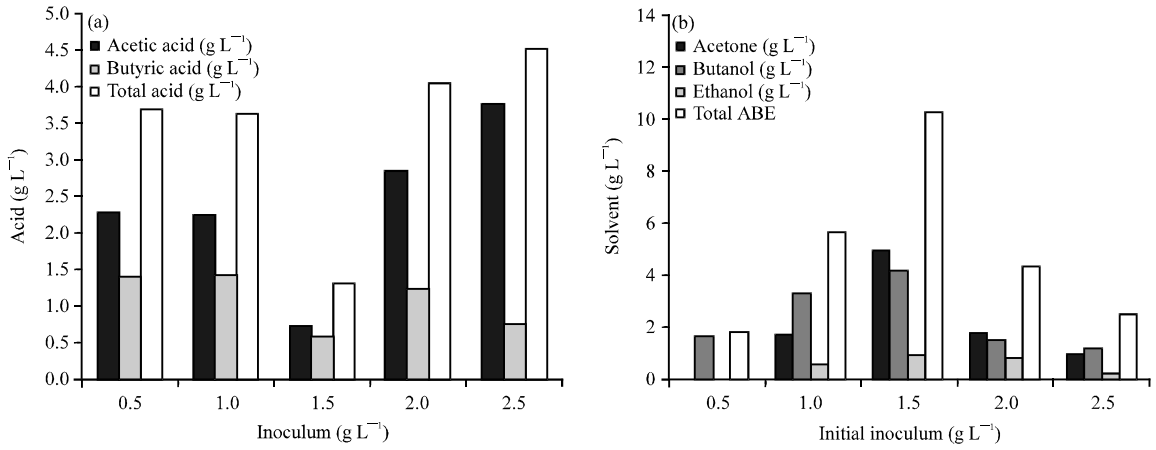

Fig. 2(a-b): (a) Total volatile fatty-acid and (b) ABE profile fermentation, ABE: Acetone, butanol and ethanol

concentration (beyond 1.5 g L^{-1}), resulted in higher amount of TVFA; 4.09 and 4.55 g L^{-1}, respectively for 2 and 2.5 g L^{-1} inoculum concentration, respectively (Fig. 2a). The ABE also followed the same pattern, the highest ABE (11.45 g L^{-1}) was recorded with the hydrolysate inoculated with inoculum concentration of 1.5 g L^{-1} (Fig. 2b). Hydrolysates inoculated with higher inoculum concentration (2 and 2.5 g L^{-1}) had lower yield and productivity (Table 2), this observation agreed with what was reported by Ferchichi *et al.* (2005). The very low ABE and TVFA values of hydrolysates inoculated with low inoculum concentration (0.5 and 1.0 g L^{-1}) may be due to low cell/substrate ratio (discussed earlier). The low cell/substrate ratio was responsible for the low content of the available glucose needed for conversion into fermentation products (TVFA and ABE), thus, limitation of glucose might have been responsible for the low yield and productivity in such hydrolysates. However, for hydrolysates inoculated with higher inoculum concentration, due to the unique nature of most microorganisms, the transformation of substrates into fermentation products during fermentation process is accompanied by some metabolic activities by the cells, these activities extract energy and carbon from the substrate to produce new biomass which ultimately also undergo microbial growth. The cells undergo major changes during these metabolic activities and this action results in a lot of negative impact on the fermentation process and its products. In addition, it is possible that the cells are unable to attain complete growth within the hydrolysates which may otherwise be due to factors like nutritional inhibition or some other unfavourable conditions. The greater the number of cells available for these activities, the more devastating the adverse effect will be on the hydrolysates. Thus, it is observed in this study that at higher inoculum concentrations beyond 1.5 g L^{-1}, despite the higher quantity of microorganisms in the hydrolysates which normally should have favored production of higher quantity of ABE, the yield and the productivity of the hydrolysate were low (Table 2). The highest TVFA value (4.55 g L^{-1}), was obtained from the medium inoculated with the highest inoculum concentration (2.5 g L^{-1}) and this produced one of the lowest ABE (2.8 g L^{-1}) in this study. This confirms that formation of acids occasioned by high initial cell density inhibits the formation of solvents as suggested by Lee *et al.* (2009) in an earlier work. The result of the one-way ANOVA statistical analysis of the effects of variation of inoculum concentration showed a significantly positive effect ($p<0.05$) on both yield and productivity of ABE (Table 3 extracted from one-way ANOVA in SPSS statistical test). The

standard deviations (Table 2) of the data for both yield and productivity showed that the average data set used did not deviate so much from the original values obtained in the actual experiments.

The changes in the metabolic pathways, which is a function of the initial cell density and the nature of the microorganism is exhibited in the relationship between ABE yield and TVFA i.e. when the TVFA is low, ABE yield is high and vice-versa (Fig. 2a, Table 2). Although, there are limited information on activities of *Clostridium saccharoperbutylacetonicum* N-14 with respect to inoculum concentration in fermentation process with ricebran as substrates, however, the above observations are in agreement with the result of previous studies by Carvalho and Curtis (1999) which indicated that low inoculum concentration causes reduced cell growth rate which invariably led to low productivity of products (Carvalho and Curtis, 1999). In addition, in an earlier study by Neelakandan and Usharani (2009), they reported that the amount of ethanol obtained from cashew apple juice increased with the inoculum concentration until it reached a maximum and thereafter decreased with further increment in inoculum concentration (Neelakandan and Usharani, 2009).

The investigations carried out here are parts of preliminary investigations into how yield and productivity of fermentation products especially butanol can be improved upon. Further experiments with respect to how this can be achieved will be conceptualized, designed and carried out in the course of the programme.

CONCLUSION

The yield and productivity of fermentation products obtained from ricebran is affected by the initial ricebran (substrate) concentration and the initial inoculum concentration. The result of one-way ANOVA statistical analysis further showed that variations of both inoculum and substrate concentrations had significant effects on the yield and productivity of ricebran fermentation products. ABE yield and productivity of ricebran hydrolysates increased linearly with inoculum concentration until it reached its maximum at 1.5 g L^{-1} cell density. Further increment in inoculum concentration led to decline in the yield and productivity of ABE. Production of higher yield and productivity of ABE is accompanied with production of lower quantity of total acids and vice-versa. The best initial RB concentration for the production of ABE through batch fermentation using *C. saccharoperbutylacetonicum* N1-4 is 100 g L^{-1} while the best inoculum concentration is 1.5 g L^{-1}. Trichloroacetic acid-an organic acid was successfully used as pretreatment agent for lignocellulosic material.

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