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BIOTECHNOLOGICAL STUDIES ON BIOFUEL PRODUCTION FROM AGRICULTURAL WASTES

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ABSTRACT: Fruits peels, an agricultural waste discarded in huge amounts, were effectively fractionated into its oligosaccharides composition to dedicate their added values utilization. Four fruits peels (Banana, Watermelon, Orange and Mango) were studied. The starch, pectin, hemicellulose, celluloses, lignin and proteins fractions were determined in terms of dry weights percentages for these peels. Mango peels showed the highest oligosaccharides levels, even lignin content was highest by 17.25%. Also, banana peels showed high oligosaccharides levels with the lowest level of lignin by 4.82%. Lab-scale bioreactor was carried out for enzymes production and saccharifaction, the banana and mango peels were released the greatest saccharified pentose and hexose sugars, the total fermentable sugars were 27.77 and 21.13 $g.l^{-1}$, respectively. Co-fermentation were conducted by selected yeast strain belong to *Kluyveromyces marxianus to contribute* previously sexual regenerative *Saccharomyces cerevisiae* for bioethanol co-production. As expected, co-fermentation increased the productivity by more than 18%, the substantial bioethanol yield were observed by saccharifed banana peels with 10.74 $g.l^{-1}$, the adding of CaO lead finally to 97.5 wt % of pure bioethanol by duplicate the distillation process. The reaction molar ratio of cocked oil to ethyl acetate were established by 0.1, 0.125, 0.2, 0.25, 0.5 and 1.0 $mol.mol^{-1}$ respectively. The highest reaction conversion was attained at temperature reaction of 60 °C, molar ratio of oil to ethyl acetate at 1 : 6 ratio (0.17 $mol.mol^{-1}$) within 6 hours of reaction time, and catalyst concentration of 0.5 *wt* %.

Key words:* *Biofuel; Biodiesel; Agro-peels; fungi co-cultivation; yeast co-fermentation.

INTRODUCTION

Fuel consumption is increasing sharply despite the apparent lack of conventional fossil fuel supplies, whether in big urban or rural areas. The excessive fuel combustion has contributed significantly to generating high levels of pollution, which led to greenhouse gas emissions, global warming severity and unpredictable climate turmoil (Jihad *et al*., 2018). Among them, raw agro-wastes which include fruits peels and vegetables residuals are a rich source of fibrous and fermentable sugars (Sharma, 2006). The huge amounts of consumed fruits and vegetables has encouraged the expand using of their generate residues to improve their simultaneously and economical biofuels competitiveness (Cutzu and Laura, 2007). Actually, the enzymatic hydrolysis is more promising than any others physicochemical hydrolysis since the reduction of utility costs, also it has the highest productivity. In the enzymatic hydrolysis, the fungi *Aspergillus niger* the most commonly industrial used, the commercial available enzymes mixtures are produced by various *Aspergillus* species which produced a broad range of polysaccharides degrading enzymes (Hu

et al., 2011). The technique of fungal co-cultivations have been previously represented for the specific degrading enzymes production, the ascomycete phytopathogen *A. niger* with the basidiomycete white rot fungus *Phanerochaete chrysosporium* could improve lignin-olytic, cellulolytic and hemicellulosic enzymes production (Kusumaningtyas *et al*., 2014), this enzymatic treatment would also avoid initially physical or chemical treatments for the easily sugars recover without much inhibitions (Verma and Madamwar, 2002). On the other hand, the yeasts are commonly used for alcoholic fermentation, mainly belonging to *Saccharomyces* genus, among other species of genus *S. cerevisiae* is usually considered the typical fermentation species, where they are preferably characterized by the highest ethanol tolerance and productivity (López-Malo *et al*., 2013). Unfortunately, the ideal yeast type which can be efficiently utilizing both C_5 and C_6 sugars has not been isolated. Almost 90% of current theoretical bioethanol yield produced by *S. cerevisiae*, it cannot ferment the pentose sugars such as the xylose present in hemicellulose which are converted into toxic furfural (Farias *et al*., 2017). Recently, many researchers have investigated the ability of simultaneously cultivate another ethanologens yeast strain with *S. cerevisiae*, which are grown and fermented together in the same reactor, these type of co-fermentation processes are became wide spread to be effective for fermenting both glucose and xylose sugars for high yield bioethanol production, the microbial co-fermentation were decreased the initial investment and maintenance costs (Yadav *et al*., 2011). The strain of *K. marxianus* is economically advantageous that are able to metabolize a wide variety of sugars via homoethanol pathway, although it require a higher cells number than *S. cerevisiae* to produce comparable bioethanol amount (Widmer *et al*., 2009). Besides, biodiesel fuel can also be applied in conventional diesel engines without making any modification in the machine. Biodiesel is generally made of methyl esters of fatty acids, which can be produced via the two main routes, i.e.. the base-catalyzed reaction of cocked oil triglyceride and/or acid-catalyzed trans-esterification (Komintarachat *et al*., 2015). The two classical routes of biodiesel production has draw back in term of the by-product resulted in the reaction, trans-esterification of triglyceride with short chain alcohols result in a large amount of glycerol as by-product, meanwhile the esterification of free fatty acid yields water as by-product. Both glycerol and water by-products are handled as waste. Chemical inter esterification of oils with methyl/ethyl acetate will result in methyl esters (biodiesel) and triacetin, whereas glycerol would not be produced in this reaction. Triacetin is well known as fuel bio-additive which can increase fuel quality in terms of viscosity, oxidation stability, and cloud flow properties such as cloud point and pour point (Maddikeri *et al*., 2013).

MATERIALS AND METHODS

1. Agricultural wastes preparation

Four different fruits peels (Banana) *Musa acuminate*, (Watermelon) *Citrullus lanatus*, (Orange) *Citrus aurantium* and (Mango) *Mangifera indica* were collected. Minced samples were oven dried at 70 °C for 48 h and grind to be used as source of fermentable sugars. While, the tissues samples were stored in darkness airtight containers at room temperature until bio-chemical analysis. The dry weight (DW) was determined by oven dried of 5 grams of each fresh fruit peel at 105 °C for not less than 2 hours or to mass constant.

2. Microbial strains and culture media

The fungal and yeasts strains used in

this study are *A. niger* ATCC® 64974™, *P. chrysosporium* ATCC® 24725™, *K. marxianus ATCC*® 36142™ and *S. cervisiae* ATCC® 9763™ brought from the national Mircen (Microbiological Resource Center, Ain Shams University). On the other side, laboratory cultures of *S. cervisiae* and *K. marxianus* strains were routinely maintained on sodium acetate ascospore agar (SAA, Himedia) for creating new generation by ascospores germination (McClary *et al*., 1959). The regenerative strains were screened to use as bioethanol co-producers based on (+20% glucose, xylose fermentative and alcohols tolerance). While, the glucose peptone agar medium, (GPA, Difco) was used for examining the new yeasts spontaneous mutant selected from the ascospores sexual conjugation.

3. Cultivation conditions and fermentation setup

For fungi maintenance and spores inoculums preparation, spore production on MEA medium slants usually requires minimum 72 h of growth at 40 °C, spores (conidia) were suspended in 5 *ml* of sterile water and then filtrated through sterile glass wool to avoid presence of fungus mycelia. Spores concentration were determined by measuring the absorbance at 650 nm (ABS 1 cm^{-1} = 5x10^6 $spores.ml^{-1}$). Fungi were co-cultivated by inoculating 1 *ml* of diluted spores suspension (5x10^5 $spores.ml^{-1}$) with a distance of 3 *cm* or by mixing the spores and inoculating them in the centre or over the whole surface of the Petri dishes. For all liquid cultures, fungi were grown on 250 *ml* shaking Erlenmeyer flasks with 40% filling volume, 250 RBM agitation rate and 0.0163 S^{-1} coefficient volumetric oxygen transfer rate (k_La) (Fahim *et al*., 2013).

4. Degrading enzymes assays and quantification

For the cellulase enzyme activity, the methods of filter paper (Whatman no.1) activity was applied (Dubois *et al*., 1956). β-glucosidase activity was determined by using 5-carboxy methyl cellulose (10 $g.l^{-1}$) as carbon replacement source in ACM media (Camassola and Dillon, 2012). Xylanase activity was determined under the same conditions by supplying ACM media with (1 $ml.l^{-1}$) extracted wheat bran solution (Saddler and Mes-Hartree, 1984). While, the liberated reduced sugars from cellulose as (glucose) and (xylose) from hemicellulose in the latest reactions mixtures were measured by the phenol-sulfuric acid methods which were used later to determine the monomers sugars extracted from the oligo-saccharides. Each one $\mu mol.min^{-1}.ml^{-1}$ of released glucose or xylose were expressed as one unite of cellulase, β-glucosidase and xylanase enzymes respectively under specified experi-mental conditions.

5. Cooked oil and biodiesel production processes

Cooked oil amounts were collected in brown glass bottles and ethyl acetate (commercial, Egypt) were the main reactants for the biodiesel synthesis. Potassium hydroxide was utilized as catalysts for the chemical inter esterification reaction. Neutralization of cooked oil was conducted in a 500 *ml* three-necks flask using sodium carbonate. Soda ash (Na_2CO_3) was initially dissolved into aqueduct to obtain saturated aqueous solution of sodium carbonate. Subsequently, 100 *ml* of cooked oil was heated to 70 °C in a three necks flask equipped with a glass condenser. After the desired temperature was attained, a solution of Na_2CO_3 was added to the cooked oil and stirred for 60 minutes using magnetic stirrer. During the neutralization process, saponification reaction of FFA contained in the oil with alkali took place and resulted in soap, then neutralized cooked oil was separated from the soap using

mechanical filtration (Komintarachat *et al*., 2015).

RESULTS AND DISCUSSION

1. Agro-west peels composition

The primarily results of biochemical analyses for four different fruits peels examined are presented in (Table 1). The percentages on dry weight base of total proteins, starch, pectin, lignin, cellulose and hemicellulose contents were investigated.

The mango peel showed the highest levels of starch, pectin, hemicellulose and lignocelluloses comp-onents by 13.56%, 11.89%, 14.51% and 51,48% respectively with highest level of lignin content by 17.25% and lowest level of total proteins by 3.89%. Also, the peel of banana showed high levels of starch, hemicellulose and lignocelluloses comp-onents by 13.27%, 12.93 % and 38.14% respectively with the lowest level of lignin contents by 4.82%, the proteins and pectin contents were in the moderated levels by 5.12% and 11.21%, respectively. While, the orange peel showed the lowest levels of starch, hemicellulose and lignocelluloses comp-onents by 3.22%, 9.27% and 30,76% respectively with 6.53% of lignin content; but the pectin content recorded the highest level by 16.45%, the proteins levels were moderate by 5.23 %, which close to the report published by Aravantinos-Zafiris and co-workers (1994). Citrus peels had somewhat lower hemicellulose and lignocellulose content as well. In the same order, the watermelon peel showed the highest level of total proteins by 8.84%. The pectin, hemicellulose and lignocellulose contents were in moderate levels by 11.34%, 11.45% and 33.84% respectively with 9.87% and 11.34% of lignin and pectin contents respectively. These results match with the reports of Green and their co-workers (2015), that banana and mango peels could be a low cost and rich dietary fibers source which composed mainly from hemicelluloses and lignocelluloses oligosaccharides (Berardini *et al*., 2005; Li *et al*., 2010). Considering these results, the peels of banana and mango seems to be good alternative of carbon sources suitable for conversion of bioethanol, these pre-ference is due to the respected hexose as well as pentose sugars converted from the good levels of starch, cellulose and hemicellulose respectively, in addition the ability of tested fungi for reducing the relatively amounts of lignin during the pre-treatment processes and fermentation process. While, this view is supported by Ververis and their co-workers (2007).

Table 1: Biochemical analyses of examined agro peels waste based on dry weight percentage (% w/w).

Agro peels wastes	Protein	Starch	Pectin	Lignocellulose		Hemi-cellulose
				Lignin	Cellulose	
Bp	5.12 ± 0.5	13.27 ± 0.2	11.21 ± 0.4	4.82 ± 0.5	33.32 ± 0.4	12.93 ± 0.3
Mp	3.89 ± 0.4	13.56 ± 0.8	11.89 ± 0.3	17.25 ± 0.1	34.23 ± 0.4	14.51± 0.8
Wp	8.84 ± 0.6	4.36 ± 0.7	11.34 ± 0.4	9.87 ± 0.3	26.71 ± 0.7	11.45 ± 0.1
Op	5.23 ± 0.8	3. 22 ± 0.1	16.45 ± 0.5	6.53 ± 0.6	24.23 ± 0.5	9.27 ± 0.6

The values are mean of three replicates ± standard deviation

2. The co-culture motivation of degrading enzymes

The better set of oligosaccharides degrading enzymes of each fungus specie were conducted for achievement the optimum condition of submerged fermentation at the presence of other fungus. The fungi co-cultivation affect were investigated by influence the oligosaccharides reduction amounts by degrading exo-enzymes, the selected cultivation conditions were also achieved during fungal species were grown together on ACM culture media supplied with wheat bran, and compared with the same conditions on single fungus culture. The data presented in (Table 2) were illustrated the activities of three oligosaccharide enzymes cellulase, β-glucosidase and xylanase enzymes as they had previously been remarked to be produced by each fungus strains.

Overall, the co-cultivation was resulted an increasing in enzymes activities by 5.8, 8.8 and 81.3 *nmol.min^{-1}.ml^{-1}* for β-glucosidase, celulase and xylanase respectively. Although, *A. niger* showed high enzymes activities compared with *P. chrysosporium*, but the activities of co-cultivation are still higher than to each single fungus culture. These positive co-operation effect between industrial ascomycete *A. niger* and the *P. chryso-sporium* basidiomycete fungus has been previously recorded by Aravantinos-Zafiris and co-workers (1994).

Either, the total secreted proteins in co-culture media (0.65 *mg.ml^{-1}*) were also stronger than those resulted by individual culture. The fungi mixed culture does not resulted a big difference in secreted proteins amounts, but it was triggered the stimulation of the specific enzymes. The xylanase and ligininase enzymes importance is centered on its ability for enhancing lignocelluloses degradability by observed its synergistic effect along with cellulase enzymes by facilitating its accessibility plus its capability for swelling of fibers and porosity increasing (Meijer *et al.*, 2011). Also, the other mode of action of the ligninase enzyme originated from the formation of fragile notches with continuous cracks in the fibers surfaces, this notches allow to the cellulase multi enzymes complex to begin from these patches, then exo-enzymes can act randomly action in synergism (Gupte and Madamwar, 1997).

3. Saccharification and bioethanol production

The process of enzymatic degradation have been followed for achieved the effectually oligosaccharides hydrolyze into fermentable sugars (Table 3), the saccharifation sugars yields and bioethanol production were varied among the type of supplemented peels into production medium. Also, the bioethanol produced was depended on the type of inoculated yeast strain.

Table 2: Total secreted protein and enzymes activities of *A. niger*, *P. chrysosporium* and its co-cultivation.

Fungi strains	Total proteins (*mg.ml^{-1}*)	Enzyme activities (*nmol.min^{-1}.ml^{-1}*)		
		Cellulase	β-glucosidase	Xylanase
A. niger	0.59 ± 0.03	7.9 ± 0.17	4.2 ± 0.13	72.8 ± 0.26
P. chrysosporium	0.42 ± 0.03	6.2 ± 0.13	3.8 ± 0.15	5.3 ± 0.24
Co-cultivation	0.65 ± 0.04	8.8 ± 0.16	5.8 ± 0.19	81.3 ± 0.23

The values are mean of three replicates ± standard deviation

Table 3: Total saccharified sugars and bioethanol yields of *A. niger*, *P. chrysosporium* and its co-fermentation.

Saccharified fruits peels	Total enzymatic released sugars $g.l^{-1}$			Produced ethanol $g.l^{-1}$ by yeasts		
	Pentose sugars*	Hexose sugars*	Mixed sugars*	*S. cervisiae*	*K. marxianus*	Co-fermentation
Bp	6.08 ± 0.5	22.13 ± 0.3	27.77 ± 0.6	9.19 ± 0.4	7.67 ± 0.6	10.74 ± 0.7
Mp	5.39 ± 0.3	16.26 ± 0.8	21.13 ± 0.5	5.62 ± 0.3	4.89 ± 0.8	6.75 ± 0.6
Wp	5.26 ± 0.2	14.47 ± 0.5	19.56 ± 0.2	6.07 ± 0.6	5.36 ± 0.2	7.35 ± 0.3
Op	4.08 ± 0.6	12.05 ± 0.2	15.97 ± 0.8	4.45 ± 0.3	3.89 ± 0.5	5.31 ± 0.4

The values are mean of three replicates ± standard deviation
***Pentose, hexose and mixed sugars (as xylose, glucose and with fructose mixture, respectively).**

The banana and mango peels were showed the greatest saccharification of pentose and hexose sugars, the total fermentable sugars from them were reached to by 27.77 and 21.13 $g.l^{-1}$ respectively. In case of watermelon peels, the released fermentable sugars were found to be at moderated level by 19.56 $g.l^{-1}$, while orange peels were produced 15.97 $g.l^{-1}$ at the lowest level of total fermentable sugars. Therefore, the bioethanol yield produced by single *S. cervisiae* fermentation was the best by 9.19, 6.07, 5.62 and 4.45 $g.l^{-1}$ from saccharified peels of banana, water-melon, mango and orange respectively. As expected, the co-fermentation were showed an increasing in bioethanol productivity by more than 18% as average percentage for all saccharifed fruits peels. The maximum bioethanol productivity was obtained from saccharified banana peels by 10.74 $g.l^{-1}$. Although, the mango peels were founded to composite a good yield of oligo-saccharides which they converted into a good amount of fermentable sugars, but the presence of high percentage of lignin components may resulted an microbial inhibitors derive-atives as well orange peels. These results were matched with those obtained by Reddy and co-workers (2003), which they have reported that, the mango peels released good amounts of fermentable sugars, but the ethanol yield was very low by direct fermentation. The bioethanol azeotropic separation process was coupled by addition of calcium oxide as drying agent which lead finally to achievable 95.5 *wt* % of pure bioethanol. Its clear that, the bioethanol distillation process could be lead finally to produce anhydrous ethanol which observed in the theoretical maximum was 95.5 *wt* %, to remove the remaining water, duplicate process should be applied to reach anhydrous ethanol 97.9 *wt* % (Li *et al.*, 2010). Among several expanding fruits peels ferment-ation is well known worldwide, other saccharifed peels fermentation is extremely rare, but it is possible due to the high sugar content of these agro peels residuals. Until yet, the ferment-ation of these peels has fundamentally been treated for lignin free residuals due to economical and

technical considerations; therefore, much more of fruit residuals that is discarded as waste could become economical substrates for bioethanol production (Banerjee *et al*., 2010).

4. Cooked oil and biodiesel production processes

Neutralization process was done for chemical refining of high free fatty acid (FFA) cooked oils types. The soap produced is then separated from the oil feedstock, the neutralization process was performed using sodium bicarbonate. Alkaline neutralization (saponification) of FFA in cooked oil with (Na_2CO_3) resulted amounts of carbonic acid H_2CO_3, these amounts were neutrally dissociate to CO_2 gas and H_2O. Carbon dioxide gas forms foam in the soap, so that the soap will float on the oil, then soap therefore can be easily separated using mechanical filtration method. The rudiment of the refined cooked oil after neutralization process was 72%, the FFA content of the neutralized oil was 0.17% or it was identical to 0.3506 $mg.g^{-1}$ of KOH cooked oil. This acidity value could fulfil the requirement for the reaction of oil using alkaline catalyst which ranged from zero to 1.0 *wt*%. Subsequent to the neutralization step, cooked oil was used as the feedstock for the biodiesel synthesis via alkaline inter esterification, Figure (1).

The results of the experiment were demonstrated in Fig (1), also showed the effect of catalyst concentration on the biodiesel yields during the alkaline-catalyzed inter esterification of cooked oil with ethyl acetate was investigated at the minimum reaction time and the normal temperature 60 °C, and the molar ratio of cooked oil to ethyl acetate by 0.1, 0.125, 0.2, 0.25, 0.5 and 1.0 $mol.mol^{-1}$ was varied. It was disclosed that biodiesel yield decreased with the increasing potassium hydroxide concentration. As depicted in Fig (1), an increase in catalyst concentration from 0.5 to 1.0 *wt* % oil brought about a corresponding reduction in the biodiesel yield from 98.9 to 79.2 %. It means that catalyst concentration of 0.5 % was the optimum point. These data were matched with Maddikeri and co-workers (2013), which reported the similar phenomenon in the inter esterification of waste cooking oil and methyl acetate in the presence of potassium methoxide catalyst. The decreasing yield comes about since the excessive amount of catalyst will enhance the formation of an emulsion, the emulsion leads to the formation of gels, which lowers the fraction of fatty acid and ethyl ester in product mixture (Suppalakpanya *et al*., 2010). Molar ratio of reactants is a significant aspect influencing the yield of biodiesel in the inter-esterification reaction, this reaction requires three moles of ethyl acetate for each mole of triglycerides, it indicates that the molar ratio of 1:3 (0.34 $mol.mol^{-1}$) is necessitated for a stoichio-metric reaction. Nonetheless, inter esterification reaction is a reversible reaction which usually need an excess of ethyl acetate above the stoichio-metric requirement to shift the equilibrium towards the right (Sousa *et al*., 2010). The effect of molar ratio of triglycerides in cooked oil to ethyl acetate was also evaluated, the other variables were maintained constant at the reaction temperature, reaction time, and catalyst concentration of 60 °C during 6 h and 0.5%, respectively. Also, the experimental results which were obtained based on the presented data in Fig (1), it was exhibited that the yield of biodiesel diminished with the increasing molar ratio of cooked oil to ethyl acetate from 1.0 (1:1) to 0.1 (1:10) $mol.mol^{-1}$, it occurred since the amount of ethyl ester (biodiesel) produced during this inter esterification reaction was far less than

the amount of un-reacted ethyl acetate. Thus, the higher amount of ethyl acetate employed in the reaction, the smaller fraction of biodiesel in the product mixture will be found. Based on the data achieved from all the experiments conducted, it was shown that the best yield was 98.9 %, provided by the inter esterification reaction performed at the molar ratio of cooked oil to ethyl acetate by 0.17 *mol.mol*$^{-1}$ (1:6) with catalyst concentration of 0.5% at normal temperature of 60 °C during 6 hours of time reaction. Biodiesel yield obtained through this inter esterification can be enhanced by optimizing the process variables, the main aspect to be evaluated is the catalyst employed in the reaction, the utilized KOH which was dissolved in ethyl acetate reactant. Since there was no alcohol in this inter esterification reaction, the active catalyst compound of potassium alkoxide can't be formed. Meanwhile, the catalytic activity of potassium oxide is significantly lower than alkoxide. Thus, for the further experiment, a small amount of alcohols (methanol/ethanol) should be utilized to dissolve potassium hydroxide and form alkoxide which will perform as an active catalyst for inter esterification reaction. Besides, the low yield was also attributed to the existence of side reaction between potassium oxide and ester forming potassium acetate. Hence, the addition of a small amount of alcohol will convert potassium hydroxide into potassium methoxide and prevent the formation of potassium acetate (Casas *et al*., 2010). The pervious investigations for the product mixture analysis obtained at the same reaction condition was found that, the amounts detected of ethyl acetate, methyl decanoat and methyl oleate were minimized by passing the process time towards triacetin amounts.

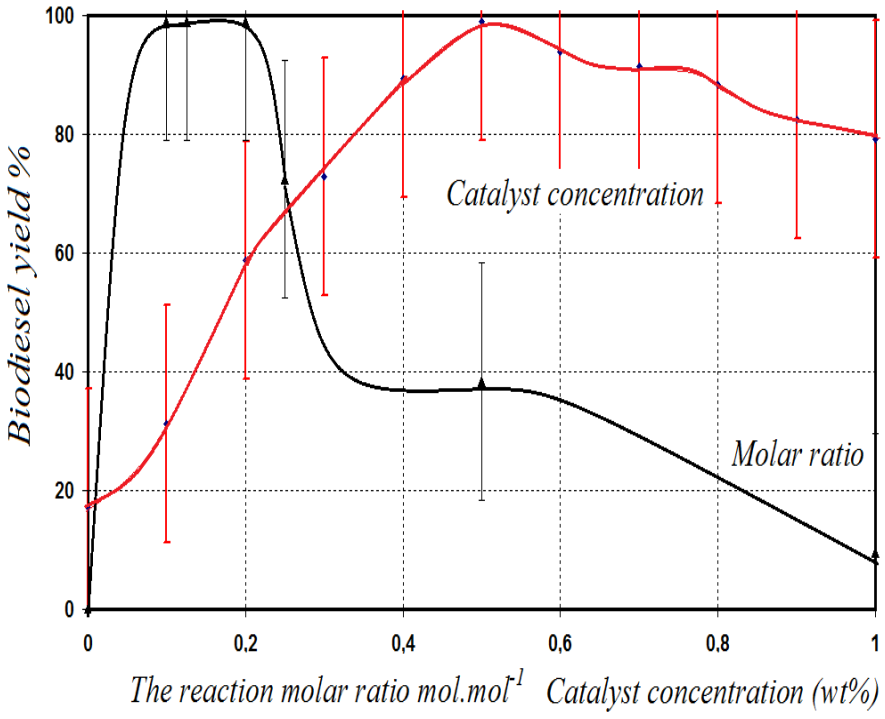

Fig (1): Effect of the reaction molar concentrations & catalyst concentration on the biodiesel yield

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REFERENCES

Aravantinos-Zafiris, G., V. Oreopoulou, C. Tzia and CD Thomopoulos (1994). Fibre fraction from orange peel residues after pectin extra-ction. *Lebensm-Wiss u-Technol*, 27: 468-471.

Banerjee, S., S. Mudliar, R. Sen, L. Giri, D. Satpute, T. Chakrabarti and R.A. Pandey (2010). Commerci-alizing lignocellulosic bioethanol: Technology bottlenecks. *Biofuels Bioprod. Biorefining*, 4, 77-93.

Berardini, N., M. Knödler, A. Schieber and R. Carle (2005). Utilization of mango peels as a source of pectin and polyphenolics. *Innov. Food Sci. and Emerg. Technol.* 6(4): 442-452.

Camassola, M. and AJP Dillon (2012). Cellulase determination: modi-fications to make the filter paper assay Easy, Fast, Practical and Efficient. *Open Access Scientific Reports*, 1: 125.

Casas, A., M.J. Ramos and A. Perez (2010). New Trends in Biodiesel Production: Chemical Interesteri-fication of Sunflower Oil with Methyl Acetate, *Biomass and Bioenergy*, 35(5): 1702-1709.

Cutzu, R. and L. Laura Bardi (2017) Agricultural Wastes Using Resi-dual Thermal Energy of a Co-generation Plant in the Distillation Phase. *Fermentation*, 3: 24, 1-8.

Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith (1956) Colorimetric method for de-termineation of sugars and related substances," *Anal. Chem.*, 28(3): 350-356.

Fahim, S., K. Dimitrov, F. Gancel, P. Vauchel, P. Jacques and I. Nikov (2013). Oxygen transfer in three phase inverse fluidized bed bioreactor during biosurfactant production by *Bacillus subtilis*. *Biochem. Engineer. J.*, 76: 70-76.

Farias, D., D.I.P. Atala and F.M. Filho (2017). Improving bioethanol production by *Scheffersomyces stipitis* using retentostat extra-ctive fermentation at high xylose concentration. *Biochemical Eng. Journal*, 121: 171-180.

Green, E.R., M.E. Himmel, GT Beckman and Z. Tan (2015). Glycosylation of cell-ulose: Engineering better enzyme for biofuels. *Adv Carbohyd Chem Biochem*, 72: 63-112.

Gupte, A. and D. Madamwar (1997). Solid state fermentation of lignocellulosic waste for cellulase and β-glucosidase production by co-cultivation of *Aspergillus elli-pticus* and *Aspergillus fumigates*. *Biotechnol. Progress*, 13: 166-169.

Hu, HL, J Van den Brink, BS Gruben, HAB Wösten, JD Gu and RP DeVries (2011). Improved enzyme pro-duction by co-cultivation of *Asp-ergillus niger* and *Aspergillus oryzae* and with other fungi. *International Biodeterioration and Biodegradation*, 65: 248-252.

Jahid, M, A. Gupta and DK Sharma (2018). Production of Bioethanol from Fruit Wastes (Banana, Papaya, Pineapple and Mango Peels) Under Milder Conditions. *J Bioprocess Biotech*, 8: 327.

Komintarachat, C., R. Sawangkeaw and S. Ngamprasertsith (2015). Con-tinuous production of palm bio-fuel under supercritical ethyl acetate. *Energy Conversion and Management*, 93: 332-338.

Kusumaningtyas, RD, PA Handayani, PS Rochmadi and A Budiman

(2014). Tin (II) chloride catalyzed esterification of high FFA jatropha oil: experimental; and kinetics study, Int. *Journal of Renewable Energy Development*, 3(2): 75-81.

Li K, S. Fu, H. Zhan, Y. Zhan and LA Lucia (2010) Analysis of the chemical composition and morphological structure of banana pseudo-stem. *BioResources*, 5(2): 576-585.

López-Malo, M., A. Querol and JM Guillamon (2013). Metabolomic com-parison of *Saccharomyces cerevi-siae* and the cryotolerant species *S. bayanus* var. *uvarum* and *S. kudriavzevii* during wine ferment-ation at low temperature. *PLoS ONE*, 8: 60135.

Maddikeri, GL, AB. Pandit and PR. Gogate (2013). Ultrasound assisted inter-esterification of waste cooking oil and methyl acetate for biodiesel and triacetin production, *Fuel Processing Technology*, 116: 241-249.

Meijer, M., JA Houbraken, S. Dalhuijsen, RA. Samson and RP. de Vries (2011). Growth and hydrolase profiles can be used as characteristics to distinguish *Aspergillus niger* and other black *Aspergilli*. *Stud. Myco.*, 69(1): 19.

McClary, DO, WL Nulty and GR Miller (1959). Effect of potassium versus sodium in the sporulation of *Saccharomyces*. *J. Bacteriol.*, 78: 362-368.

Reddy, GV, PR. Babu, P. Komaraiah, Roy KRRM and IL. Kothari (2003). Utilization of banana waste for the production of lignolytic and cellulolytic enzymes by solid substrate fermentation using two *Pleurotus* species (*P. ostreatus* & *P. sajorcaju*). *Proc. Biochem*, 38: 1457-1462.

Saddler, JN and M. Mes-Hartree (1984). The enzymatic hydrolysis and fermentation of pretreated wood substrates. *Biotechnol. Advances*, 2(2):161-181.

Sharma, DK (2006). Bioprospecting for drug research and functional foods for prevention of diseases-Role of flavonoids in drug development. *J Sci Ind Res*, 2: 391.

Sousa, LL, IL Lucena and FAN Fernandes (2010). Transesterification of castor oil: Effect of the acid value and neutralization of the oil with glycerol, *Fuel Proces. Tech.*, 91(2): 194.

Suppalakpanya, K., S. Ratanawilai and C. Tongurai (2010). Production of ethyl ester from esterified crude palm oil by microwave with dry washing by bleaching earth, *Applied Energy*, 87(7): 2356.

Verma, P. and D. Madamwar (2002) Pro- duction of ligninolytic enzymes for dye de-colorization by co cultiv-ation of white rot fungi *Pleurotus ostreatus* and *Phanerochaete chrysosporium* under solid state fermentation. *Appl. Bioch. and Biotechnology*, 102: 109-118.

Ververis, C., K. Georghiou, D. Danielidis, DG Hatzinikolaou, P. Santa, R. Santas and V. Corleti (2007). Cell-ulose, hemicelluloses, lignin and ash content of some organic materials and their suitability for use as paper pulp supplements, *Bioresource Technology*, 98: 296.

Widmer, WW, JA Narciso, K. Grohmann and MR Wilkins (2009). Simulta-neous saccharification & ferment-ation of orange processing waste to ethanol using *Kluyvero-myces marxianus*. *Biol Eng*, 2:17-29.

Yadav, KS, S. Naseeruddin, GS Prashanthi, L Sateesh and LV Rao (2011). Bioethanol fermentation of concentrated rice straw hydrolysate using co-culture of *Saccharomyces cerevisiae* and *Pichia stipitis*. *Bioresour Technol*, 102: 6473-6478.

دراسات بيوتكنولوجية على انتاج الوقود الحيوى من المخلفات الزراعية

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الملخص العربي:

كان الهدف من هذا البحث هو دراسة إمكانية استخدام المخلفات الزراعية في إنتاج الوقود الحيوي وذلك عن طريق بعض المعاملات التكنولوجية الخاصة. حيث تم اختيار أربعة أنواع من قشور الفاكهة وهى الموز والبطيخ والبرتقال والمانجو لإجراء التجارب عليها. وقد تم تقدير محتوى هذه المخلفات من النشا ، البكتين ، الهيميسيلولوز ، السليلوز ، اللجنين والبروتينات من حيث نسب الأوزان الجافة لهذه القشور. وقد أظهرت النتائج علي إن قشور المانجو سجلت أعلى مستويات من البوليسكريدات، كما أن محتوى اللجنين كان أعلى بنسبة ١٧,٢٥٪. أيضا ، وقد أظهرت قشور الموز مستويات عالية من البوليسكريدات، ولكن مع أدنى مستوى من اللجنين بنسبة ٤,٨٢ ٪. في حين ، تم استخدام اثنين من سلالات الميكروبية *[illegible]* للتنمية المشتركة بينهم. حيث أظهرت الفحوصات الإنزيمه أن التنمية المشتركة يمكن أن تحسن إفراز الأنزيمات الخارجية. وقد نتج عن التنمية المشتركة زيادة في أنشطة هذه الأنزيمات بنسبة ٥,٨ و ٨,٨ و ٨١,٣ نانومول.الدقيقة$^{-١}$.مل$^{-١}$ لكل من الزيلينيز و سيليوليز وبيتا جلايكوسيديز على التوالي. وقد تم تنفيذ مفاعل حيوي معملي لإنتاج الإنزيمات والتخمير، وأنتجت قشور الموز والمانجو اعلي كمية من السكريات الخماسية والسداسية. وكان إجمالي السكريات المخمرة منها ٢٧,٧٧ و ٢١,١٣ جم$^{-١}$.لتر$^{-١}$ على التوالي. أجريت عملية التخمير المشترك بواسطة سلالة مختارة من الخميرة المستنبطة جنسيا تنتمي إلى كلفيروميسيز ماكزينيس للإسهام إنتاج البيوإيثانول. كما هو متوقع ، زادت عملية التخمير المشترك من ناتج الإيثانول الحيوي بأكثر من ١٨٪ كمتوسط لجميع القشور المختبرة . كما تم تقدير ناتج البيوإيثانول الناتج عن طريق قشور الموز ب ١٠,٧٤ جم–١ ، وعند إضافة أكسيد الكالسيوم كعامل تجفيف أدي في النهاية إلى زيادة درجة النقاوة قدرت ب ٩٧,٥٪ بالوزن من البيوإيثانول النقي خاصة عند تكرار عملية التقطير. كما تم في هذا العمل، تقييم تأثير العوامل الرئيسية الداخلة في تفاعل الاسترات المشتركة في إنتاج البيوديزل. كما تم دراسة تأثير تركيز المادة المحفزة في المدى من صفر إلى ١,٠٪ مقدرة للوزن. كما تم اختبار نسب التركيز المولاري من زيت الطهي المستخدم إلى أسيتات الإيثيل المضافة بنسبة تتراوح من ٠,١، ٠,١٢٥ ، ٠,٢ ، ٠,٢٥ ، ٠,٥ و ١,٠ مول.مول$^{-١}$ على التوالي. حيث تم التفاعل عند درجات الحرارة المعتادة للتفاعل وهى ٦٠ درجة مئوية، ونسبة مولارية من الزيت إلى أسيتات الإيثيل بنسبة ١: ٦ (٠,١٧ مول.مول$^{-١}$) خلال ٦ ساعات من كوقت التفاعل الأمثل، وكذلك تركيز المحفز وهو ٠,٥٪ مقدرا للوزن.

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