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Bioconversion of Potatoes to Bioethanol

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Abstract*. With the depletion of the world´s petroleum supply, there has been an increasing worldwide interest in alternative, non-petroleum based sources of energy. Ethanol derived from biomass has the potential to be renewable transportation fuel that can replace gasoline. Moreover bioethanol can play an important role in reducing green-house gas emission. Ethanol use will increase because of its biodegradable, renewable and performance qualities. It is a high performance fuel in internal combustion engines and burns relatively cleanly, especially as the amount of gasoline with which it is blended decreases. The largest potential feedstock for ethanol includes materials such as agricultural residues, forest residues, wood, grass, waste paper and municipal wastes. In the present work, potatoes were used as example of starch-based biomass. Different variables were studied for their effect on the percent of bioethanol produced as a result to saccharification and fermentation of the raw material, which included: quantity of enzyme (α-amylase and/or Aspergillus niger (AN), addition of yeast (Saccharomyces cerevisiae (SC)), temperature during fermentation, separate saccharification and fermentation (SHF) and simultaneous saccharification and fermentation (SSF). The maximum percent of alcohol was 56% (b.w.) at 40°C when 3g of α-amylase and 8g of yeast were added using SSF strategy. A comparison between obtained results and published data and strategies were presented.*

Keywords*: bioethanol, biofuels, potatoes, biomass, ethanol*

1.Introduction

In recent years increasing interest in the production of biofuels especially bioethanol as an alternative to fossil fuels has gained considerable attention in various countries particularly Brazil and the U.S.A. [1]. Depletion of petroleum-based fuels worldwide, with the concomitant rise in its prices has led to the search for substitutes other than petroleum for production of energy. In this context, bioethanol is currently produced from a variety of starch-based crops such as potatoes, rice, corn, wheat, and cassava [2] lignocellulosic biomass which includes agricultural residues (e.g., corn stover and crop straw), herbaceous crops (e.g., switchgrass), forestry residues and municipal solid wastes (e.g., waste paper) [3].

Ethanol production from biomass consists of several consecutive steps, which include pretreatment, hydrolysis, fermentation and product separation. Pretreatments are necessary to improve the digestibility of the lignocellulosic biomass. The main effects are dissolving hemicellulose and alteration of lignin structure by providing an improved accessibility of the cellulose for hydrolytic enzymes [4]. The hydrolysate contains varying amounts of monosaccharides, both pentose and hexose, and a broad range of substances either derived from raw material or resulting as reaction products from sugar and lignin degradation. Many of these substances may have an inhibitory effect on the microorganisms in subsequent fermentation steps. The fermentation organism must be able to ferment all monosaccharides present and in addition, withstand potential inhibitors in the hydrolysates. The most commonly used ethanol producers, SC, cannot ferment pentoses, which may constitute up to 45% of the raw material. Among the xylose fermenting yeasts Pichia stipitis has shown promise for industrial applications because it ferments xylose rapidly with a high ethanol yield [5].

Numerous overviews and reviews have been published concerning bioethanol production from

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liognocellulosic wastes. Chandel et al. [6] provided a broad overview on current status of bioethanol production technologies in terms of their economic and environmental viability. In this regard, Ardjmand et al. [7] reviewed the conversion of lignocellulosic wastes to bioethanol with a focus on pretreatment technologies to increase enzyme accessibility to biomass and yields of fermentable sugars. Lee [8] reviewed the important key technologies required for the successful biological conversion of lignocellulosic biomass to ethanol. Saini et al. [9] reviewed the various steps involved in the production of the second-generation bioethanol. Bhatia et al. [10] reported the future trends for costs reduction in the production of bioethanol. Kumar et al. [11] discussed the various pretreatment methods, including alkali-, physical-, physicochemical- and chemical- pretreatments.

Abouzied and Reddy [12] investigated the direct fermentation of unhydrolyzed potato starch to ethanol by monocultures of the amylolytic fungus, AN, and cocultures of AN and SC. They found that the amylolytic activity, rate and amount of starch utilization, and ethanol yields, increased several-fold in coculture. Khan et al. [13] investigated the use of potato wastes as source of bioethanol, in which the effect of *p*H, temperature and mixture of digesting enzymes during saccharification and fermentation on the production was examined. Tasić et al. [14] investigated the hydrolysis of starch from tubers by hydrochloric and sulphuric acids, at different ratios of plant material to acid solution. Rani et al. [15] used flour prepared from potato tubers after cooking and drying, for ethanol production. Liquefaction of potato flour slurry was carried out with α-amylase followed by saccharification with glucoamylase. Akponah and Akpomie [16] investigated the feasibility of bio-ethanol production from yam, potato and cassava root peels. Slurry of each peel was saccharified using acid, commercially available α-amylase and AN. Liimatainen et al. [17] investigated three methods for the determination of ethanol content in waste potatoes firstly by a method based on density of sample, secondly, a method based on distillation and density measurement of sample, and thirdly a method based on gas chromatography.

The present work investigates the possibility of producing bioethanol from pretreated (cleaned/ washed) potatoes by saccharification and fermentation with α-amylase and yeast respectively. Numerous factors were studied for their effect on the amount of ethanol produced, expressed as concentration of the produced bioethanol in solution with water. Samples during experimenting were analyzed by determination of the density at different time intervals from which the concentrations were obtained from relevant tables in Handbook for Chemical Engineers [18]. The obtained results compared with other published papers in the field.

2.Materials and methods

Potatoes obtained from the local market, were used as the raw materials for the production of bioethanol. ***AN*** was prepared in the lab in order to function as a source of cellulase enzyme. Yeast (***SC)*** was purchased from the market in packets, and α-amylase used was a product of Sigma, USA. First, the potatoes were washed, dried, peeled, cut into pieces, weighed to the second decimal place on an analytical balance, ground in a special lab-grinding machine, then emptied in a 1L beaker and tap water added with the same weight as potatoes, according to Table 1, covered tightly, and the whole mash was boiled for 1h at constant volume. The samples incubated at temperatures 35, 40 and 45ºC to choose the optimum temperature for fermentation.

Table 1. The conditions of all experiments conducted for ethanol production using potatoes

Exp. no.	mass of potatoes (g)	Water volume (mL)	room temperature (°C)	α -amylase (g)	mass of yeast (g)	*Aspergillus niger* area (*AN*)(cm²)	temperature of fermentation (°C)	SHF	SSF	Notes	minimum density (g/mL)	maximum alcohol % (wt%)
1	413.14	413	20	1	16	-_	35	✓	_-	SHF with α-amylase	0.9385	38.3
2	232.77	233	20	1.12	9	-	35	✓	-_	SHF with α-amylase	0.9728	17
3	203	203	20	0.98	7.9	-_	35	✓	-_	SHF with α-amylase	0.9772	13
4	211	211	20	1.02	8.2	1	35	✓	-_	SHF with α-amylase &*AN*	0.916	49

5	214.4	215	25	1.04	8.3	1	35	-_	✓	SSF with α-amylase &*AN*	0.9202	45
6	214.4	215	20	1.04	8.3	1	45	-__	✓	SSF with α-amylase &*AN*	0.9628	24.2
7	221.2	221	20	1.07	8.6	1	45	-__	✓	SSF with α-amylase &*AN*	0.9148	49.6
8	203.3	203	25	0.98	7.9	1	40	_-__	✓	SSF with α-amylase &*AN*	0.9199	45.45
9	207.2	207	20	1	8	2	35	_-__	✓	SSF with α-amylase e & doubling of *AN*	0.94	37.6
10	406	406	25	0.98	15.7	1	45	-__	✓	SSF with α-amylase &*AN*	0.9186	46
11	400	400	25	0.97	15.5	1	40	-	✓	SSF with α-amylase & AN	0.9328	39.4
12	200	200	20	2	8	-	40	-	✓	-	0.9354	40
13	200	200	20	3	8	-	40	-	✓	-	0.9006	56
14	200	200	20	2	16	-	40	-	✓	-	0.9395	37.8
15	213	213	20	1.03	16.5	1	40	-	✓	-	0.9363	39.45
16	250	250	20	3	8	-	35	-	✓	-	0.951	31.6
17	210	210	20	3	8	-	45	-	✓	-	0.97	18.98
18	350	350	20	1	8	-	40	-	✓	-	0.9142	49.84
19	350	350	20	2	8	-	40	-	✓	add 1g α-amylase to exp.18 (consec. exps.)	0.9446	35
20	150	150	20	1	8	-	40	-	✓	-	0.925	44.9

Table 1 shows the conditions of all the experiments conducted in the present work, which includes experiment number, mass of potatoes, water volume, lab temperature, α-amylase quantity, mass of yeast, area of AN, fermentation temperature, time required for minimum density, the mode of experimentation whether SHF or SSF, and the *p*H was set in the range (4-5) as reported by Ramesh et al. [19].The mash was left to cool to 35°C (or otherwise), and the *p*H was then measured and adjusted to 4.5-5. 1g of α-amylase (or otherwise), according to Table 1, was added to the mash and stirred at the desired temperature. 1cm^2 of AN, was cut into tiny pieces then added to the previous constituents, stirred at constant temperature, and the beaker covered once more with cling wrap. 8g of SC according to Table 1 were dissolved in 100mL of water at 35°C while stirring, then added to the contents of the beaker (using SHF or SSF strategy) at the desired temperature according to Table 1 (e.g. 35°C). The mixture was then heated while stirring to the required temperature for 1hr, according to Table 1, then a sample of the centrifuged and filtered mash suspension was taken to determine its density. After weighing on an analytical electrical balance to the fourth digit, the density was determined, and the percent concentration by weight of ethanol was determined from tables found in Handbook for Chemical Engineers [18].

3.Results and discussions

Table 1 presented earlier, clarifies the conditions of all the experiments conducted in the present work. The effect of the different factors that have been investigated in this work are presented in the following paragraphs.

Effect of fermentation temperature

The effect of temperature during fermentation under different conditions as regards quantity of α-amylase, yeast, and AN, on the time required to attain maximum fermentation of pretreated potatoes using SSF strategy is illustrated in Figure 1. It shows that 45^{o}C is the most suitable fermentation temperature among the temperatures investigated.

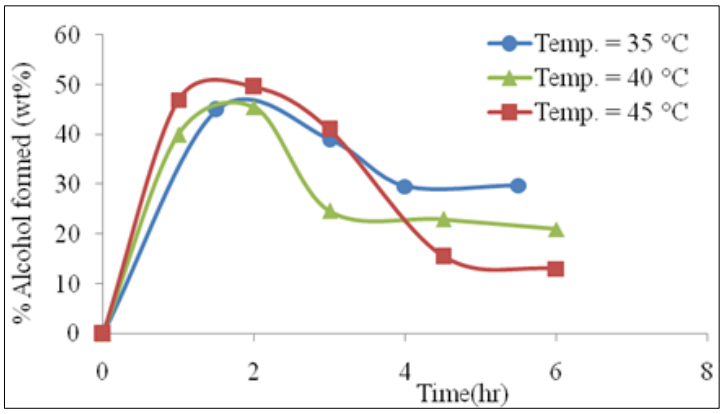

Figure 1. Effect of fermentation temperature on the percent of ethanol formed from potatoes, 1g α-amylase, 8 g yeast, 1cm^2 AN, using SSF strategy

Occasionally, centrifugation was used for accelerated separation of the bioethanol solution from the very fine particles, when separation was tedious and time-consuming in order to avoid partial evaporation of ethanol on prolonged filtration and in case the liquid sample contained fine particles which clogged the filter paper. The maximum percent alcohol concentration achieved in this case was 49.6. This result has been obtained by Khan et al. [13] for the production of bioethanol through enzymatic hydrolysis, the data of their study of potato revealed that significant % bioethanol was produced at high temperature. This might be due to the inappropriate temperature condition contributing to the lack of metabolic activity which consequently had an effect on the diffusion of substrate and product. It is worth noting that SSF took place in the three aforementioned experiments. Thus, α-amylase takes care of converting amylose to glucose, the latter is then fermented with yeast to bioethanol. AN was added to assist in saccharification of any lignocellulose that might be present from incompletely peeled potatoes.

Figure 2 clarifies a similar comparison for two experiments conducted at 40 and 45°C at different conditions indicated in the figure caption, in which the SSF strategy was applied. It shows that the quantity of yeast was doubled keeping the α-amylase and AN the same as before. The results, once again show that lower density and therefore higher percent alcohol took place at 45°C compared to 40°C. However, the result emphasizes the fact that the bottle-neck is the saccharification step and not the fermentation, since despite that the yeast was doubled and which is responsible for fermenting the produced sugar, yet the restricted amount of monosaccharide formed made the extra added yeast invaluable.

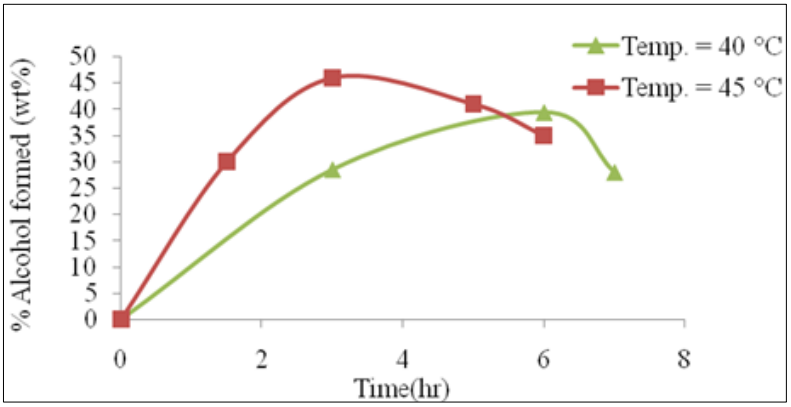

Figure 2. Effect of fermentation temperature on the percent of ethanol formed from potatoes, 1g α-amylase, 16 g yeast, 1cm^2 AN, using SSF strategy

Therefore, values were more or less comparable to those in which the yeast was half the present quantity. However, it is worth noting that the addition of α-amylase should have been coupled with glucoamylase in order to hydrolyse the amylopectin portion of the potato starch and lead to all the starch being hydrolysed. However, we could not find any glucoamylase in the chemicals market and had to use α-amylase alone.

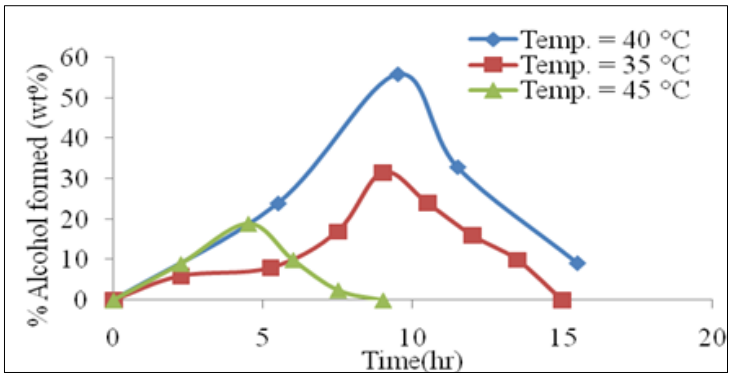

Figure 3. Effect of fermentation temperature on the percent of ethanol formed from potatoes, 3g α-amylase, 8 g yeast, without AN, using SSF strategy

Accordingly, inspecting Figure 3 proves that maximum concentration of bioethanol was produced at 40°C, while the concentration dropped to the thereabouts of 19%. Moreover, the minimum density took place after 10 h elapsed, while maximum concentration (56%) was achieved at 40°C, the latter results may be probably due to the excess enzyme which might have led to a higher saccharification of the amylose fraction, which together with the absence of AN, allowed efficient contact between the amylose and enzyme. This observation was recorded by Khawla et al. [1] since their results indicated that the higher the enzyme concentration was, the higher the fermentable reducing sugar content.

Effect of strategy type

Comparison between SSF and SHF as regards density of bioethanol solution and percent alcohol formed is depicted in Figure 4, which proves that SHF is preferred to SSF, especially that the reactions were conducted at 35°C. Moreover, the optimum temperature for fermentation of sugars to bioethanol was around 45°C. Accordingly, conducting saccharification initially at 35°C gives the opportunity to the amylose fraction to be totally converted to monosaccharides especially that the dope is devoid of yeast and AN, which if present, may have competed with the amylase in the available area subjected to the amylose.

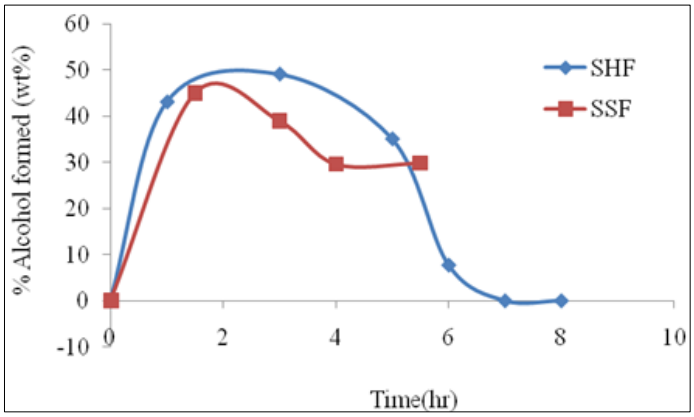

Figure 4. Effect of strategy type on the percent of ethanol formed from potatoes, 1g α-amylase, 8 g yeast, and 1cm^2 AN, fermentation temperature 35°C

Moreover, in the case of SSF strategy, owing to the aforementioned fact, the saccharification is expected to be less than that taking place in case of SHF, therefore less bioethanol is expected to be formed in case of SSF. However, the difference between the two techniques is narrow (49 and 45% bioethanol) in case of SHF and SSF respectively. In this regards, a similar close difference in results between the two techniques was recorded by Rani et al. [15].

Effect of mass of yeast

In starch, contrary to other lignocellulosic biomass, potato pulp is poor in hemicellulose and lignin, suggesting that it could be saccharified by common yeast, without the need for an engineered yeast capable of fermenting xylose, and it could be treated without delignification process [20].The effect of SC mass on the concentration of the alcohol formed using SSF, at 40°C is illustrated in Figure 5 from which, it is clear that when 8g of yeast were used percent alcohol reached 40%, whereas on doubling the yeast (16g), it led to decreasing the concentration to 37.8%.

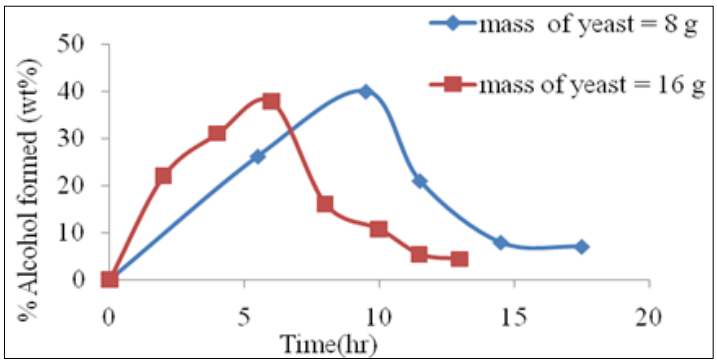

Figure 5. Effect of yeast mass on the percent of ethanol formed from potatoes, 2g α-amylase, fermentation temperature 40°C, without AN, using SSF strategy

However, the small difference may be attributed to a yeast overdose, since increasing the yeast dosage is not expected to give a deleterious effect; on the contrary, it should push the reaction (fermentation) forward.

One final remark is that the concentration of bioethanol is not one of the best results despite that α-amylase was doubled to 2g which may have been attributed to the temperature during SSF being 40°C. It is worth noting that as the yeast dosage increases, the time required for the completion of fermentation decreases. This observation was ascertained by Abouzied and Reddy [12], similar to our case in Figure 5.

Figure 6 clarifies a similar comparison for two experiments conducted at 40°C, using AN and SSF strategy, from which it is clear that when 7.9g of yeast were used, percent alcohol reached 45.45%, whereas using 16.5g of yeast resulted in a lower concentration of 39.45%. The results, once again show that higher alcohol percent took place at 8g of yeast, however, the result emphasizes that increasing the yeast dosage gave a lower alcohol concentration, while simultaneously using 1g of α-amylase instead of using 2g of α-amylase, as in the aforementioned comparison.

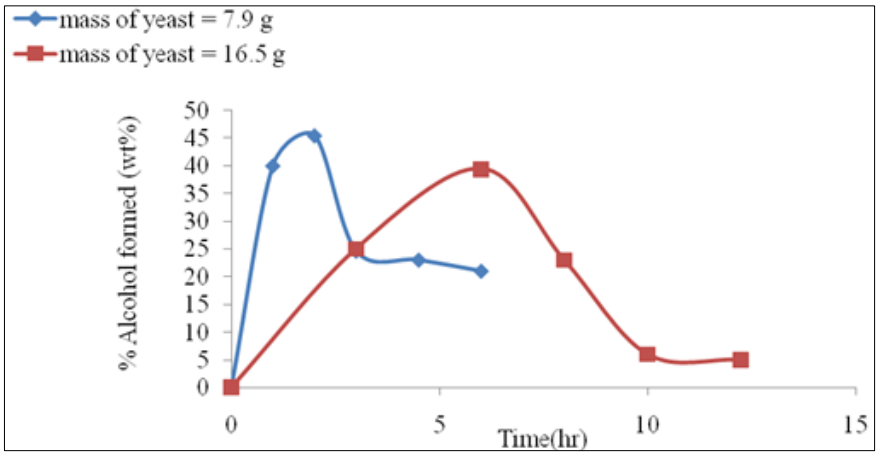

Figure 6. Effect of yeast mass on the percent of ethanol formed from potatoes, 1g α-amylase, fermentation temperature 40°C, 1cm^2 AN, using SSF strategy

Effect of α-amylase dose

The effect of increasing the α-amylase on the alcohol concentration formed using SHF strategy at 35°C in the absence of AN is illustrated in Figure 7, from which it is clear that it had a deleterious effect, which was not expected; on the contrary, it should have pushed the reaction (fermentation) forward, but it is observed here, that when using half amount of α-amylase, the percent of bioethanol was 38%, whereas on doubling the amount of α-amylase, it led to decreasing the concentration of bioethanol to 17%. However, a significant difference is observed, since increasing the α-amylase dosage is not expected to give a lower alcohol concentration as the concentration of α-amylase increases, but the time required for the completion of fermentation decreases.

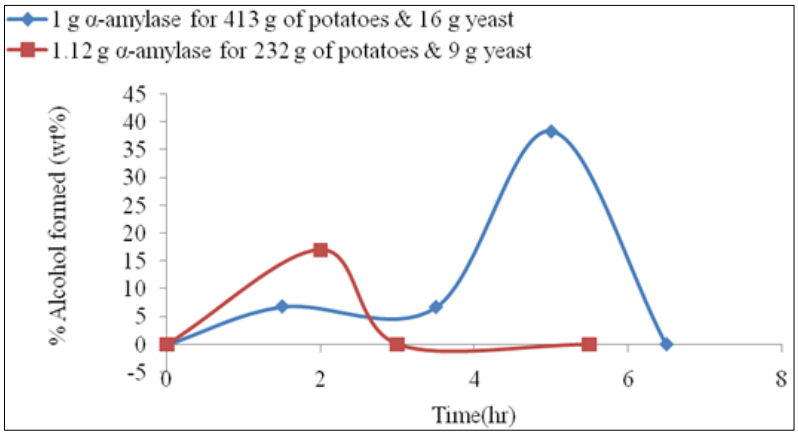

Figure 7. Effect of α--amylase dose on the percent of ethanol formed from potatoes, fermentation temperature 35°C, without AN, using SHF strategy

Under other different conditions, the effect of α-amylase was also illustrated in Figure 8. It is clear that experiments were carried out in the absence of AN using SSF strategy. It was noticed that increasing the α-amylase to 3g (triple the amount) led to an increase in the bioethanol formed (56%), while doubling the α-amylase dosage (2g) gave 40% of bioethanol formed. It was expected that tripling the α-amylase amount might lead to an increase in the bioethanol formed due to the probable insufficiency of the enzyme leading to incomplete saccharification of the amylose, which means that the excess of enzyme might have led to a higher saccharification of the amylose fraction, which accompanied with the absence of AN allowed efficient contact between the amylose and enzyme, This observation is in agreement with

Khawla et al. [1] since their results indicated that the higher the enzyme concentration was, the higher the fermentable reducing sugar content.

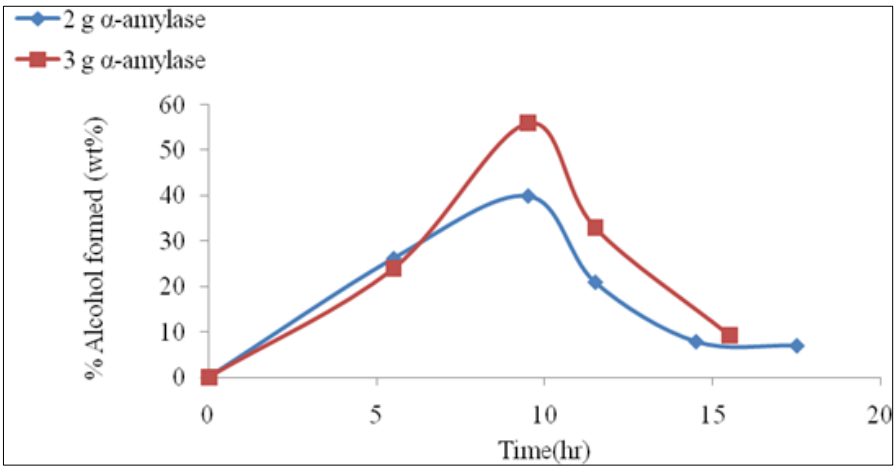

Figure 8. Effect of α-amylase dose on the percent of ethanol formed from potatoes, 8g yeast, fermentation temperature 40°C, without AN, using SSF strategy

Effect of AN area

Coculture of AN and SC was selected for this study because AN consistently gave higher ethanol yields than other cocultures [12]. The effect of AN area under certain conditions is clarified in Figure 9; the conditions are stated in the figure caption. AN was added to assist in saccharification of any lignocellulosic materials that might be present from incompletely peeled potatoes, the experiments were carried out at 35°C. From the figure it is noticed that doubling the dosage of AN led to a decrease in the percent of bioethanol formed (37.6%), whereas conducting the experiment using 1cm^2 of AN, keeping the other conditions constant, gave a higher percent of bioethanol (45%). Accordingly, it is clear that doubling of AN amount was less effective than using half of the amount, since it was not required, and only contributed in preventing the proper access of reactants to each other for reaction.

Effect of increasing temperature for continued experiments on two consecutive days

An experiment was conducted to see the effect of increasing the temperature from 35 to 45°C for continued experiments, on two consecutive days, on the density and hence the concentration of bioethanol formed. Figure 10 clarifies this effect, from which, it is obvious that when the experiment was carried out at 35°C under the mentioned conditions, the percent of bioethanol reached 45%, whereas heating the same solution on the following day at 45°C gave a bioethanol percent of 24.2%. Accordingly, raising the temperature from 35 to 45°C gave the opportunity to the glucose fraction which remained to be totally converted to bioethanol which confirms that the optimum fermentation temperature of sugars to bioethanol was around 45°C.

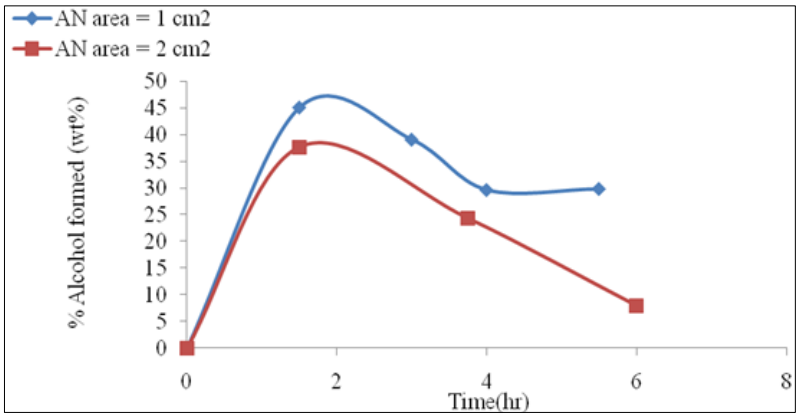

Figure 9. Effect of AN area on the percent of ethanol formed from potatoes, 1g α-amylase, 8g yeast, fermentation temperature 35°C, using SSF strategy

Effect of increasing α-amylase dosage for continued experiments on two consecutive days

Another attempt for continued experiments on two consecutive days was conducted to see the effect of increasing α-amylase dosage from 1 to 2 g, in the absence of AN using SSF strategy at 40°C. This effect is illustrated in Figure 11.

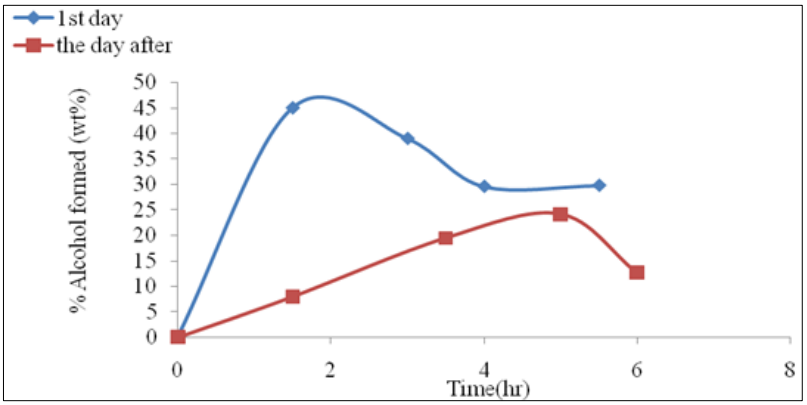

Figure 10. Effect of increasing temperature from 35 to 45°C for continued experiments on two consecutive days on the percent of ethanol formed from potatoes, 1g α-amylase, 8g yeast, 1cm^2 AN, using SSF strategy

The results show that the percent of bioethanol was 49.84% for 1g α-amylase, while the percent of bioethanol reached 35% when another gram of α-amylase was added to the same solution. Accordingly, the percent of bioethanol decreased from 49.84 to 35%, when the α-amylase amount was doubled. Another remark is that, increasing the α-amylase dosage gave the opportunity to the unconverted amylose fraction to be totally converted to glucose (monosaccharide), then converting the latter to bioethanol, thus the addition of enzyme increases the process of bioethanol production and enhances conversion of starch into sugar [13].

Effect of coupling AN with α-amylase

As the use of enzyme combination was necessary for effective hydrolysis [1], the effect of coupling AN (as a source of cellulase) with α-amylase was studied and shown in Figure 12 under definite conditions which are stated in the figure caption, from which, it is clear that coupling of AN with α-amylase achieved lower density and therefore higher percent alcohol (49%). Moreover, in case of using AN only, the percent of bioethanol reached 13%. A significant difference is observed between the two

cases. It can be said that coupling of AN with α-amylase enhances the conversion of all the starchy and lignocellulosic materials present in the solution, thus improving the bioethanol formation. This may be due to the fact that the coculture of AN and α-amylase prevents the accumulation of inhibitors, accordingly improves the conversion of sugars to bioethanol. This observation was recorded by Abouzied and Reddy [12], and was also confirmed by Kongkiattikajorn and Sornvoraweat [21, 22-24].

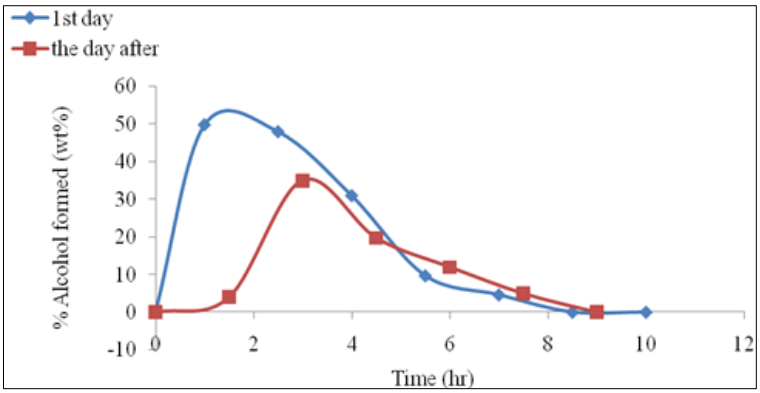

Figure 11. Effect of increasing α-amylase from 1 to 2 g for continued experiments on two consecutive days on the percent of ethanol formed from potatoes, 8g yeast, fermentation temperature 40°C, without AN, using SSF strategy

Effect of doubling both α-amylase and yeast at the same time

Figure 13 clarifies the effect of doubling both α-amylase and yeast together. The results show that doubling the quantity of both α-amylase and yeast gave a maximum percent of bioethanol of 44.9%, whereas using half the quantity used, gave a maximum percent of 49.84 (as clarified in Figure 13).

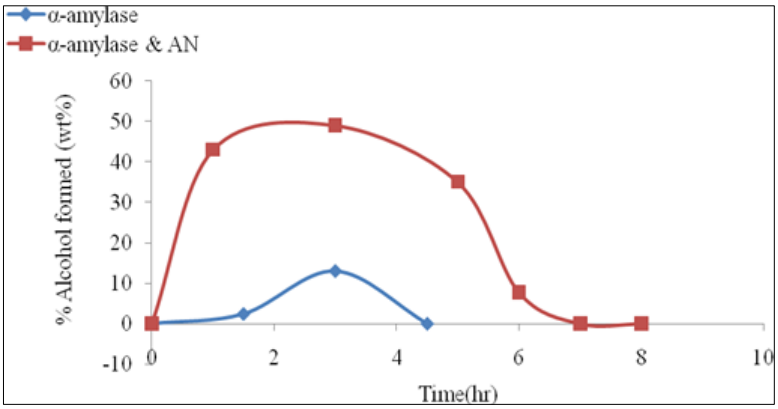

Figure 12. Effect of coupling AN with α-amylase on the percent of ethanol formed from potatoes, 1g α-amylase, 8 g yeast, fermentation temperature 35°C, using SHF strategy

Thus, the difference between the two cases is narrow, although it was expected that doubling both α-amylase and yeast would have a great effect on both saccharification and fermentation processes. However, once again it seemed to have contributed in preventing the proper encounter of reactants to take place for reaction.

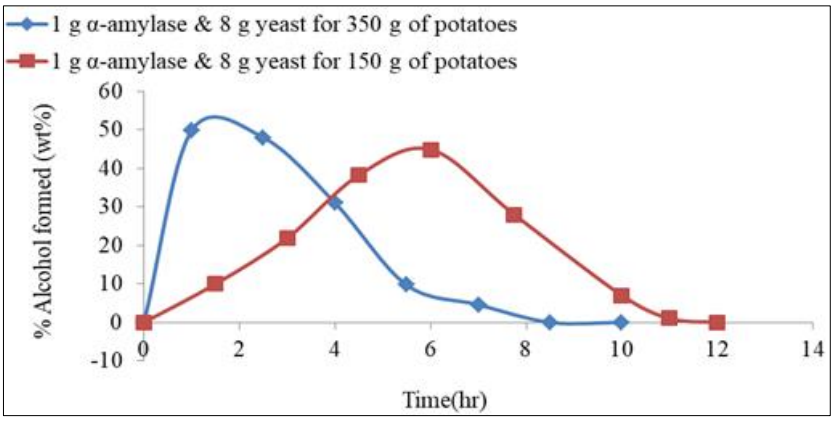

Figure 13. Effect of doubling both α-amylase and yeast on the percent of ethanol formed from potatoes, 1g α-amylase, 8 g yeast, fermentation temperature 40°C, without AN, using SSF strategy

Confirming reproducibility

In order to confirm reproducibility of experiments, the density and percent of bioethanol formed were determined twice under same conditions as shown in Figure 14 from which it is clear that in the first trial the percent bioethanol reached 17%, while in case of reproducible trial, the percent bioethanol formed reached a comparable value of 13%. Accordingly, the results can be considered reproducible.

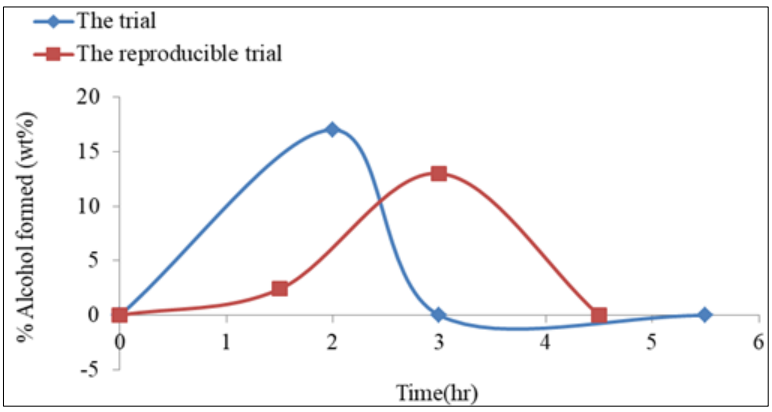

Figure 14. Confirmation of reproducibility in the preparation of bioethanol from potatoes, 1g α-amylase, 8 g yeast, fermentation temperature 35°C, using SHF strategy

4. Conclusions

From this work, the following conclusions were arrived at:

The optimum fermentation temperature is 45^{o}C.

Higher percent bioethanol is formed when SHF rather than SSF strategy is used (49 and 45% alcohol, in respective order).

The controlling step in the bioconversion of potatoes to bioethanol is the saccharification step rather than the fermenation step.

The quantity of yeast had a negligible effect on the yield of bioethanol, when SSF strategy was used, due to the control of the saccharification step in the bioconversion, being more determinant.

The higher the quantity of enzyme, in the absence of AN and using the SHF strategy led to less bioethanol formed, at 35^{o}C.

The higher the quantity of enzyme, in the absennce of AN and using the SSF strategy led to more bioethanol formed, at 40^{o}C.

The conversion of potatoes to bioethaol takes a much longer time in case of using SSF strategy at 40oC than when using SHF at 35^{o}C.

Doubling the AN gave a lower yield of bioethanol (37.6%), at 35^{o}C using SSF strategy, compared to the single dose (45%).

Increasing the temperature from 35 to 45^{o}C, on two consecutive days, produced a lower concentration of bioethanol (45 and 24.2% on 1st and 2nd day respectively) which suggests that continued operation is preferred.

Coupling of AN with α-amylase enhances the conversion of all the starchy and lignocellulosic materials present in the solution, thus improving the bioethanol yield.

Doubling both α-amylase and yeast reduced the concentration of bioethanol produced, due to improper access of reactants to each other, for reaction.

Reproducibility of the results is acceptable.

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