Introduction

Banana is an important food crop for tropical and subtropical regions and plays a crucial role in food security and the economy. According to the Food and Agriculture Organization (FAO) 2014, global banana production was around 118 million tons per year in the year 2012 and 2013. India was the leading producer, followed by China, the Philippines, and Brazil. Most of the banana produced is Musa acuminata genomes, which are sweet bananas or dessert bananas (68%), while hybrids of M. acuminata and M. balbisiana are mostly cooking bananas or plantains (32%). Unripe plantains contain high resistant starch and dietary fibers which have health benefits and attract the food industry [2]. Most plantain varieties require cooking (boiling, roasting or frying) before they can be eaten [3]. Plantain processing industries are generating huge waste in the form of unripe banana peel at one place. Unripe plantain peels weigh up to 40% of the fresh weight of the product [4]. Thus, it is indispensable to study waste management and utilization of unripe banana peel.

In today’s world, the dependency on fossil fuels for various energy purposes is increasing day by day. Many countries have mandates on ethanol blend with gasoline to be used as fuel. Ethanol is also a very good organic solvent [5-7]. Hence, ethanol needs are rising to reduce fuel imports, boosting rural economies and improving air quality. Main feedstocks for ethanol production are sugarcane and corn grains, while many other agricultural raw materials are used worldwide [7]. Most of the feedstock’s used currently for ethanol production are also used as food for human. Many attempts are being made to find new sources of feedstock, which are non-edible and easily available [7]. Currently, sugar and starch containing feedstocks are most widely used. Lignocellulosic material, however, can also be used; it is difficult to convert them into sugar, which requires advanced technology that may increase operating costs [6, 8, and 9]. Consistent effort has been made to design and improve a process, which would produce a sustainable transportation fuel using low-cost feedstocks. Many agricultural raw materials

1 Introduction
rich in fermentable carbohydrates tested worldwide for bioconversion from sugar to ethanol, but the cost of carbohydrate raw materials has become a limiting factor for large-scale production by the industries employing fermentation processes [9]. The price of feedstock contribute more than 55% to the production cost, inexpensive feedstock such a lignocellulosic biomass and agricultural food waste are being considered as alternative to expansive feedstock [10]. The production of ethanol from comparatively cheaper source of raw materials using efficient fermentative microorganisms is the only possible way to meet the demand of ethanol [11]. Agricultural and food processing industry creates huge amount of waste every year [12]. The failure or inability to salvage and reuse such materials economically results in the unnecessary waste and depletion of natural resources [12]. The solid wastes generated by fruit processing industries can serve as potential raw materials for the production of primary and secondary metabolites of industrial significance by use of microorganisms [13]. Theses fruit processing wastes can be used as potential feedstock for ethanol production and this could be an attractive alternative for disposal of the polluting residues [14, 15]. There are reports available on waste management from fruit industry waste such as; production of microbial enzymes for industrial use [12]; production of alcohol [17]; production of wine and vinegar; production of biogas [17].

The objective of present work was to study effective management and utilization of unripe banana peel waste of plantain processing waste. Dried unripe banana peel powder (DUBPP) was analyzed for chemical composition. Further, DUBPP was optimized for maximum sugar release by acid hydrolysis. Yeast strains were screened for various attributes to produce ethanol from acid hydrolysate of DUBPP. Fermentation conditions for ethanol production was optimized for acid hydrolysate of DUBPP by one factor at a time method.

2 Methods

2.1 Raw material preparation and chemical characterization of DUBPP

Unripe banana peels of hybrid variety of Musa acuminata and Musa balbisiana were collected from local banana chips manufacturer in bulk. This unripe banana peels were chopped into pieces and dried at 60°C for 24 h in tray drier then further ground to fine powder in electric grinder and sieved through mesh number 36 (0.45 mm). This raw material was stored in airtight container and kept in the desiccators until used for further experiments [18]. Moisture and ash content in DUBPP were determined as per the method of AOAC [19]. Total protein content was determined using Micro Kjeldahl procedure with a nitrogen-to-protein conversion factor of 6.25. Fat content was determined by Soxhlet method (using Instant Soxhlet apparatus-Socs Plus, Pelican equipments, Chennai, India) using petroleum ether (B. P. 60-80°C) as the solvent and carbohydrate by difference (Percentage carbohydrate content = 100 – (percentage moisture + percentage ash + percentage protein + percentage fat)). Aliquot of water soluble extract of powder sample was analysed for water soluble reduced sugar using dinitrosalicylic acid (DNS) method [20]. Starch was extracted using diluted perchloric acid and further analysed using anthrone reagent [21, 22]. Pectin content was determined using carbazole sulphuric acid method [23]. Cellulose content was determined using antherone reagent as described in Sadasivam and Manikam [23]. Estimation of neutral detergent fibre (NDF), acid detergent fibre (ADF), hemicellulose and lignin were done by gravimetric method [23, 24]. All results reported as average of three determinations.

2.2 Optimization of acid hydrolysis by one factor at time (OFAT) approach

Acid hydrolysis was done using concentrated H$_2$SO$_4$. The parameter such as temperature, acid concentration (H$_2$SO$_4$), solid loading and reaction time for acid hydrolysis was optimized by OFAT approach and details of experimental conditions are given in Table 1. The first factor was then changed until an optimum value was reached. This optimum value for the first factor was then held constant while the second variable was varied and so on. All the experiments were conducted in triplicate and standard deviation was calculated.

2.3 Maintenance of yeast strains

Three strains of S. cerevisiae (NCIM 3095, NCIM 3570 and NCIM 3059) were procured from National Chemical Laboratory, Pune, Maharashtra, India. This culture was maintained in a MYGP medium composed of malt extract 3 g, yeast extract 3 g, glucose 10 g, peptone 5 g, agar 20 g in 1000 mL distilled water. Media was autoclaved at temperature of 120°C, pressure of 100 kPa for 20 min. Sub culturing was done every month. Master culture was stored at 4°C. For seed culture, a loop full of the yeast from the slant was transferred in 100 mL of MGYP broth in 250
A.G. Waghmare, S.S. Arya

by using inverted Durham tubes as described by Subashini et al. Sedimentation characteristics of yeast strains were studied as described by Brooks [25].

2.5 Optimization of fermentation by one factor at time (OFAT) approach

Fermentation study was performed in Erlenmeyer flasks closed with rubber cork and out rubber tube was used for release of CO₂. All the experiments were carried out in triplicate using acid hydrolysate of DUBPP. Seed culture was grown in MYGP medium and aseptically inoculated in acid hydrolysate. Those flask were incubated on rotary shaker at 150 rpm. Six experimental parameters were optimized such as seed age, seed volume, fermentation time, pH, temperature and nitrogen source by OFAT approach and detail conditions are mentioned in Table 2. OFAT was implemented by varying one factor at time and keeping other parameters constant. Ethanol concentration was determined as described below and expressed as g/l; ethanol productivity is expressed as g/l/h and calculated as gram of ethanol produced in liter of hydrolysate per hour of fermentation; Conversion efficiency is expressed as % w/w and calculated as practical yield divided by theoretical yield multiplied by 100. Theoretical yield was calculated by assuming that 1 g sugar yields 0.51 g ethanol [27].

2.6 Analytical methods

Quantitative determination of reducing sugar concentration was done by DNS method [20] and ethanol concentration was analysed using gas chromatography (GC) (Chemito 8610) equipped with flame ionization detector (FID) and a column poropak-Q (6ft, ½ inch O.
3 Results and discussion

3.1 Chemical characterization DUBPP

Chemical characterization of DUBPP is given in Table 3. DUBPP contains only 2.1% w/w of water soluble reducing sugar which was not practical to utilize for fermentative production of ethanol. But, DUBPP was found to be rich source of starch (41.2% w/w) and cellulose (9.3% w/w). This carbohydrates can be easily acid hydrolyzed to fermentable sugars. Fischer et al. stated that process production of ethanol from starch sources is highly efficient, but generally starchy feedstock is costly [29]. In present paper, first time reveiling the chemical composition of DUBPP containing notable high amount of starch, cellulose and also protein. Chemical composition of DUBPP is suitable for fermentative production of ethanol after hydrolysis. Hence this fruit waste has potential to be used as biomass for production of ethanol [30].

Table 2. Various parameter used for optimization of fermentation of acid hydrolysate of DUBPP.

| Variable parameters | Constant parameters |
|---------------------|---------------------|
| Seed age (h) (6, 12, 18, 24, 30) | - | 4 | 48 | 6 | 30 | NA |
| Seed volume (% v/v) (4, 8, 12, 16) | 12 | - | 48 | 6 | 30 | NA |
| Fermentation time (h) (6, 12, 18, 24, 30, 36, 42, 48) | 12 | 12 | - | 6 | 30 | NA |
| pH (4, 5, 6, 7) | 12 | 12 | 36 | - | 30 | NA |
| Temperature (˚C) (25, 30, 35, 40) | 12 | 12 | 36 | 5 | - | NA |
| Nitrogen sources (1% w/v) (malt extract, yeast extract, peptone, urea, ammonium sulphate) | 12 | 12 | 36 | 5 | 30 | _ |

NA: Not added

Table 3. Chemical characterization of dried unripe banana peel powder (DUBPP).

| Parameters | % w/w on dry weight basis* |
|------------|---------------------------|
| Moisture   | 10.0 ± 0.00               |
| Total ash  | 7.6 ± 0.19                |
| Protein    | 8.4 ± 0.10                |
| Fat        | 4.7 ± 0.28                |
| Carbohydrate | 69.4 ± 0.14             |
| Water soluble reducing sugar | 2.1 ± 0.20 |
| Starch     | 41.2 ± 2.1                |
| Pectin     | 7.4 ± 0.68                |
| Cellulose  | 9.3 ± 0.29                |
| Hemicellulose | 3.2 ± 0.59              |
| Lignin     | 2.3 ± 0.78                |
| Acid Detergent Fibers (ADF) | 17.5 ± 0.55 |
| Neutral Detergent Fibers (NDF) | 20.7 ± 0.63 |

*Results are mean ± SD of three determinations
3.2 Effect of acid hydrolysis parameters on reducing sugar release from DUBPP

Acid hydrolysis is widely utilized in the industry for chemical hydrolysis processes due to efficiency and low cost [31, 32]. Therefore in present study, acid hydrolysis of DUBPP was carried out using H$_2$SO$_4$. Effects of various parameters on reducing sugar release are shown in Figure 1-4. It was observed that reducing sugar release increased with temperature. Optimized temperature was 120°C in autoclave at 100 kPa pressure given optimum sugar release. 1.5% v/v of H$_2$SO$_4$ was optimized for maximum sugar release further side of no increment was found. Application of high concentrations of H$_2$SO$_4$ resulted in browning or charring of hydrolysate occurred with increasing acid concentrations and also tend to formation of undesirable by-products along with sugar such as furfural and 5-dihydroxymethyl furfural, which are known to inhibit fermentation [32, 33]. These compounds are reported to be produced in very small concentration but they may be toxic to fermentation [34, 35]. In industrial scale solid loading is important parameter as it deciding factor of productivity. Then, aim was to take maximum amount of solid loading per batch. As the solid loading increased from 5 to 25% w/v sugar release decreases due to increase in viscosity which might lead to restrict the hydrolysis [36]. Solid loading (20% w/v) was taken as optimum for acid hydrolysis. Acid hydrolysis reaction time

---

**Figure 1.** Effect of temperatures (60°C, 80°C, 100°C in water bath and 120°C in autoclave at 100 kPa) on reducing sugar release at constant conditions (Reaction time: 10 min; Solid load: 5% w/v; H$_2$SO$_4$:1% v/v).

**Figure 2.** Effect of H$_2$SO$_4$ Concentrations (% v/v) on reducing sugar release at constant conditions (Reaction time: 10 min; Solid load: 5% w/v; Temperature: 120°C (under 100 kPa pressure)).
is important as it responsible for speed of overall process. Duration of reaction (20 min) was found to be optimum at that time 49.2% of sugar release was obtained under optimized conditions.

### 3.3 Selection *S. Cerevisiae* on the basis of their important attributes for production of ethanol from DUBPP

There are different problems in the fermentative production of ethanol, the most important being yeast conversion efficiency, contamination, downstream process and inhibition by high substrate and product concentrations [37]. To overcome those challenges selection of yeast was done on the basis of important attributes for ethanol production [29]. Conversion efficiency is the most important attribute of screening of yeast strains. Conversion efficiency of the yeast strain during 60h fermentation cycle was carried and shown in Figure 5. *S. cerevisiae* NCIM 3095 was the most efficient ethanol producer giving maximum conversion efficiency of 42.8% w/w at 36h in acid hydrolysate of DUBPP. The osmotolerance, ethanol tolerance, thermal tolerance, fermentation ability at high temperature and sedimentation rate of three yeast strains were studied and summarized in Table 4. Osmotolerance has been studied as high substrate, product or salt concentrations that

---

**Figure 3.** Effect of solid loading (% w/v) on reducing sugar release at constant conditions (Reaction time: 10 min; H₂SO₄:1.5% v/v; Temperature: 120°C (under 100 kPa pressure)).

**Figure 4.** Effect of reaction time (min) on reducing sugar release at constant conditions (Solid load: 20% w/w; H₂SO₄:1.5% v/v; Temperature: 120°C (under 100 kPa pressure)).
A.G. Waghmare, S.S. Arya

found to be highest thermal tolerant strain which shown growth at 45°C. Not just thermal tolerance is important for ethanol production but also need to retain fermentation ability at high temperature. Fermentation ability of yeast strains at high temperature was inspected for presence of CO₂ in inverted Durham’s tube in fermentation medium at various temperatures. All three yeast strain shown fermentation ability up to 40°C beyond that temperature, fermentation ability was lost. High sedimentation rate of yeast helps in separation of biomass and supernatant. Sedimentation property of yeast strains provide effective, environment-friendly, simple and cost effective way of cell recycles by separating yeast cells from the culture broth after in-situ sedimentation of cells in the bioreactor [39, 40]. Sedimentating yeast strain suitable for developing efficient bioprocesses to produce [30]. S. cerevisiae NCIM 3095 strain was sediment faster than other strains (see Table 4).

increase osmotic pressure are commonly encountered in industrial fermentations [29]. Osmotolerance was evaluated by varying sugar concentration from 15 to 30% w/v. S. cerevisiae NCIM 3095 strain was expressed highest osmotolerance up to 30% w/v. Ethanol tolerance is an important limiting factor for industrial exploitation of ethanol production. To investigate ethanol tolerance ability of strains, ethanol concentration was varied from 4 to 10% w/v in TOL broth. S. cerevisiae NCIM 3095 was tolerated 10% w/v ethanol concentration, whereas the other strains did not. In many warm countries, including India, summer temperatures frequently reach more than 35°C and in the typical ethanol fermentation processes carried out at 25 to 35°C with no cooling system. Due to exothermic metabolic reactions temperature rises to above 40°C leading to reduced ethanol productivities. Therefore it is very much essential to select thermo tolerant strain [38]. In present study, S. cerevisiae NCIM 3095 strain was found to be highest thermal tolerant strain which shown growth at 45°C. Not just thermal tolerance is important for ethanol production but also need to retain fermentation ability at high temperature. Fermentation ability of yeast strains at high temperature was inspected for presence of CO₂ in inverted Durham’s tube in fermentation medium at various temperatures. All three yeast strain shown fermentation ability up to 40°C beyond that temperature, fermentation ability was lost. High sedimentation rate of yeast helps in separation of biomass and supernatant. Sedimentation property of yeast strains provide effective, environment-friendly, simple and cost effective way of cell recycles by separating yeast cells from the culture broth after in-situ sedimentation of cells in the bioreactor [39, 40]. Sedimentating yeast strain suitable for developing efficient bioprocesses to produce [30]. S. cerevisiae NCIM 3095 strain was sediment faster than other strains (see Table 4).

Figure 5. Conversion efficiency (% w/w) of S. cerevisiae (NCIM 3095, NCIM 3570 and NCIM 3059) strains from acid hydrolysate of unripe banana peel powder (UBPP) to ethanol in at pH (6), shaking 150 rpm in room temperature.

Table 4. Selection S. cerevisiae on the basis of their important attributes.

| Strains     | Osmotolerance Glucose conc. (% w/v) | Tolerance to ethanol Ethanol conc. (% v/v) | Thermotolerance Temperature (°C) | Fermentation ability Temperature (°C) | Sedimentation rate (%) |
|-------------|------------------------------------|--------------------------------------------|---------------------------------|--------------------------------------|------------------------|
|             | 15  | 20  | 25  | 30  | 4   | 6   | 8   | 10  | 35  | 40  | 45  | 50  | 30  | 35  | 40  | 45  |            |
| NCIM 3095   | +++ | +++ | ++  | -   | +   | +   | +   | +   | +++ | ++  | +   | -   | ** | ** | *   | - | 93.1         |
| NCIM 3570   | +++ | ++  | +   | -   | +++ | ++  | -   | -   | +++ | +   | -   | -   | *  | *  | *   | - | 3.5          |
| NCIM 3059   | +++ | +   | -   | -   | +++ | +++ | ++  | -   | +++ | +   | -   | -   | ** | ** | *   | - | 3.1          |

Note: +++ Dense growth, ++ Medium growth, + Growth present, - Growth absent  
** Vigorous fermentation, * Fermentation present, - Fermentation absent
3.4 Effect of fermentation parameters on ethanol production from acid hydrolysate of DUBPP

All fermentation experiments were carried out in triplicate and mean results are reported in Figure 6 and Table 5. Seed age is important parameter responsible for reduction of batch time as contributes in reduction of lag phase of production batch. The seed age 12h was found to be optimum seed age as it reaches maximum conversion efficiency (49.9% w/w) at 36h (see Figure 6).

Effect of various seed volume, fermentation time, pH and temperature on ethanol concentration (g/l), ethanol productivity (g/l/h) and conversion efficiency (% w/w) of acid hydrolysate of DUBPP using S. cerevisiae NCIM 3095 is given in Table 5. As seed volume was increased from 4 to 16% v/v results increase in ethanol concentration was observed. Konar et al. suggested seed volume between 10% v/v was ideal for ethanol production [41]. So in present study, seed volume 12% v/v was selected for further optimization. Fermentation time is important parameter as it affects ethanol concentration as well as ethanol productivity. Fermentation time (36 h) was given optimum ethanol concentration as well as good ethanol productivity.

![Figure 6. Effect of seed age (6 h, 12 h, 18 h, 24 h and 30 h) on conversion efficiency (% w/w) of acid hydrolysate of unripe banana peel powder (UBPP) to ethanol at pH (6), seed volume (4% v/v), and shaking 150 rpm in room temperature by S. cerevisiae NCIM 3095.](image)

![Table 5. Effect of various fermentation conditions on ethanol concentration, ethanol productivity and conversion efficiency of acid hydrolysate of DUBPP using S. cerevisiae NCIM 3095.](table)

| Parameters               | Seed volume (%) | Fermentation time (h) | pH  | Temperature (°C) |
|--------------------------|-----------------|-----------------------|-----|------------------|
|                          | 4               | 8                     | 12  | 16               | 24 | 36 | 48 | 4   | 5   | 6   | 7   | 25  | 30  | 35  | 40  |
| Ethanol concentration (g/l) | 23.0a           | 24.5a                 | 26.8a | 27.5a          | 11.3a | 24.5a | 27.1a | 27.0a | 18.3a | 33.9a | 28.1a | 10.0a | 21.1a | 35.6a | 29.5a | 5.96d |
| Ethanol productivity (g/l/h) | 0.63a           | 0.68a                 | 0.74a | 0.76a          | 0.94a | 1.0a  | 0.75a | 0.56a | 0.76a | 1.4a  | 1.2a  | 0.41a | 0.88a | 1.5a  | 1.2a  | 0.24a |
| Conversion efficiency (% w/w) | 45.9a           | 48.8a                 | 53.5a | 54.8a          | 42.5a | 52.9a | 54.1a | 53.9a | 36.5a | 67.5a | 55.9a | 19.9a | 42.0a | 71.0a | 58.9a | 11.9a |

*The values are means from three determinations.
The values with different superscripts in a row differ significantly (p < 0.05).
Hence, fermentation time (36h) was selected as optimized fermentation time. Initial pH of acid hydrolysate was varied from 4 to 7. At pH 5 maximum ethanol concentration was obtained, similar observation is reported by Fakruddin et al. [42]. Temperature shown remarkable influence on ethanol production as temperature increased from 25 to 30°C ethanol production was significantly increased but beyond that sudden drop in ethanol production. Therefore, temperature 30°C was selected as optimized temperature. Additional nitrogen source was supplied for optimization of ethanol production. But there was not much effect was observed compared to control on ethanol production (see Figure 7). This might be due to already presence notable high protein in DUBPP. Similar finding was reported by Joshi in case of red potatoes for ethanol production [43].

4 Conclusions

The unripe banana peel is major waste of plantains processing industry. From chemical characterization reveals DUBPP is abundant source of starch, other carbohydrates and protein. *S. cerevisiae* NCIM 3095 was found best strain for production of ethanol compared to other two strains. The maximum sugar release was obtained after acid hydrolysis i.e. 49.2% w/w from DUBPP at optimized condition. The optimum conditions for fermentative production of ethanol from acid hydrolysate of DUBPP were seed age (12h), seed volume (12% w/w), fermentation time (36h), pH (5) and temperature (30°C), at this condition maximum ethanol concentration (35.6 g/l), productivity (1.5 g/l/h) and conversion efficiency (71.0% w/w) was achieved using *S. cerevisiae* NCIM 3095. The utilization of unripe banana peel waste generated from plantains processing can be done for ethanol production and this could be effective way of waste management and utilization.

Acknowledgments: The authors would like acknowledge the Department of Biotechnology (DBT), Government of India for their financial support.

Conflict of interest: Authors declare nothing to disclose.

References

[1] Food Agriculture Organization of the United Nations, FAOSTAT. Rome, Italy: FAO, 2014.
[2] Sartori T., Menegalli F.C., Development and characterization of unripe banana starch films incorporated with solid lipid microparticles containing ascorbic acid, Food Hydrocolloid., 2015, 55, 210-219.
[3] Giraldo Toro A., Gibert O., Ricci J., Dufour D., Mestres C., Bohuon P., Digestibility prediction of cooked plantain flour as a function of water content and temperature. Carbohydr. Polym., 2015, 118, 257-265.
[4] Branca C., Blasi C.D., A lumped kinetic model for banana peel combustion, Thermochim. Acta, 2015, 614, 68-75.
[5] Nasidi M., Deeni Y., Agu R., Walker G., Fermentation of stalk juices from different Nigerian sorghum cultivars to ethanol, Bioeth., 2013, 1, 20-27.

![Figure 7](image_url)  
**Figure 7.** Effect of nitrogen sources (1% w/v) on conversion efficiency (% w/w) of acid hydrolysate of unripe banana peel powder (UBPP) to ethanol at pH (5), seed volume (12% v/v), shaking 150 rpm, fermentation time 36 h and temperature 30°C by *S. cerevisiae* NCIM 3095.
Utilization of unripe banana peel waste as feedstock for ethanol production

[6] Sanchez O.J., Cardona C.A., Review: Trends in biotechnological production of fuel ethanol from different feedstocks, Bioresour. Technol., 2008, 99, 5270-5295.

[7] Brethauer S., Wyman C.E., Review: Continuous hydrolysis and fermentation for cellulosic ethanol production, Bioresour. Technol., 2010, 101, 4862-4874.

[8] Muhammad Nasidi M., Agu R., Deeni Y., Giginyu I.B., Walker G., Bioconversion of degraded husked sorghum grains to ethanol, Bioeth., 2015, 2, 1-11.

[9] Yamashita Y., Sasaki C., Nakamura Y., Effective enzyme saccharification and ethanol production from Japanese cedar using various pre-treatment methods, J. Biosci. Bioeng., 2010, 110, 79-86.

[10] Campo I.D., Alegria I., Echeverria M., Echeverria I., Diluted acid hydrolysis pre-treatment of agro-food wastes for bio-ethanol production, Ind. Crop. Prod., 2006, 42, 214-221.

[11] Pramanik K., Rao D.E., Kinetic study on ethanol fermentation of grape waste using Saccharomyces cerevisiae yeast isolated from toddy, IE (I) Journal, 2005, 85, 53-58.

[12] Essien J.P., Akpan E.J., Essien E.P., Studies on mould growth and biomass production using waste banana peel, Bioresour Technol, 2005, 96, 1451-1456.

[13] Khan N., Roes-Hill M., Welz P.J., Grandin K.A., Kudanga T., Van Dyk J.S., et al., Fruit waste streams in South Africa and their potential role in developing a bio-economy, S. Afr. J. Sci., 2015, 111, 5-16.

[14] Choia I.S., Leea Y.G., Khanalb S.K., Parkc B.J., Bae H.J., A low-energy, cost-effective approach to fruit and citrus peel waste processing for bioethanol production, Appl. Energ., 2015, 140, 65-74.

[15] Wyman C.E., Twenty years of trials, tribulations, and research progress in bioethanol technology, Appl. Biochem. Biotechnol., 2001, 91, 5-21.

[16] Hammond J.B., Egg R., Diggins D., Coble C.G., Alcohol from bananas, Bioreasur. Technol., 1996, 56, 125-130.

[17] Guneseelan N.V., Biochemical methane potential of fruits and vegetables solid waste feedstocks, Biomass Bioenerg., 2004, 26, 389-399.

[18] Oberoi HS., Vadlaniri P.V., Saida L., Bansal S., Hughes J.D., Ethanol production from banana peels using statistically optimized simultaneous saccharification and fermentation process, Waste Manag., 2011, 31(7), 1576-84.

[19] AOAC (Association of Official Analytical Chemists), Official methods of analysis, 16th ed. Washington, 2001.

[20] Miller G. L. Use of dinitrosalicylic acid reagent for determination of reducing sugar, Anal. Chem., 1956, 31, 426-428.

[21] Branyikova I., Marsalkova B., Doucha J., Branyik T., Bisova K., Zachleder V., Vitova M., Microalgae-novel highly efficient starch producers, Biotechnol. Bioeng., 2011, 108, 766-776.

[22] Takeshita T., Ota S., Yamazaki T., Hira A., Zachleder V., Kawano S., Starch and lipid accumulation in eight strains of six Chlorella species under comparatively high light intensity and aeration culture conditions, Bioresour Technol., 2014, 158, 127-34.

[23] Sadasivam S., Manikam A., Biochemical Methods 2nd ed. Chemistry and allied sciences books of new age international limited, New Delhi, India, 2005.

[24] Van Soest P.J., Robertson J.B., Lewis B.A., Method for dietary fibre, neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition, J. Dairy Sci., 1991, 74, 3583-3597.

[25] Brooks A.A., Ethanol production potential of local yeast strains isolated from ripe banana peels, Afr. J. Biotechnol., 2008, 7, 3749-3752.

[26] Subashini D., Ejilane J.,Radha A., Jayasri M.A., Suthindhiran K., Ethanol production from sago waste usingSaccharomyces cerevisiae Vits-M1, Curr. Res. J. Biol. Sci., 2011, 3, 42-51.

[27] Tan L., Sun Z.Y., Okamoto S., Takaki M., Tang Y.Q., Morimura S., Kida, K., Production of ethanol from raw juice and fresh juice of sugar beet by continuous ethanol fermentation with flocculating yeast strain KF-7. Biomass Bioenerg., 2015, 81, 265-272.

[28] Hari Krishna S., Chowdary G.V., Optimization of simultaneous saccharification and fermentation for the production of ethanol from biomass, J. Agr. Food Chem., 2000, 48, 1971-1976.

[29] Fischer C.R., Klein-Marcuschamer D., Stephanopoulos G., Selection and optimization of microbial hosts for bio-fuels production, Metab. Eng., 2008, 10, 295-304.

[30] Wu W.H., Hung W.C., Lo K.Y., Chen Y.H., Wan H.P., Cheng K.C., Bioethanol production from taro waste using thermo-tolerant yeast Kluyveromyces marxianus K21, Bioresour. Technol., 2015, 201, 27-32.

[31] Abd-Rahim F., Wasoh H., Zakaria M.H.R., Ariff A., Kapri R., Ramli N., Siew-Ling L., Production of high yield sugars from Kappaphycus alvarezii using combined methods of chemical and enzymatic hydrolysis, Food Hydrocolloid., 2014, 42(2), 309-315.

[32] Meinita M.D.N., Hong Y.K., Jeong G.T., Comparison of sulfuric and hydrochloric acids as catalysts in hydrolysis of Kappaphycus alvarezii (cottoni). Bioprocess Biosyst. Eng., 2012, 35, 123-128.

[33] Larsson S., Palmqvist E., Hahn-Hagerdal B., Tengeborg C., Stenberg K., Zacchi G., et al. The generation of fermentation inhibitors during dilute acid hydrolysis of soft-wood. Enzyme Microb. Technol., 1999, 24, 151-159.

[34] Hahn-Hagerdal B., Galbe M., Gorwa-Grauslund M.F., Liden G., Zacchi G., Bio-ethanol the fuel of tomorrow from the residues of today, Trends Biotechnol., 2006, 24, 12.

[35] Patle S., Lal B., Investigation of the potential of agro-industrial material as low cost substrate for ethanol production by using Candida tropicalis and Zyymomonas mobilis. Biomass Bioenerg., 2008, 32(7), 596-602.

[36] Sharma S.K., Enzymatic saccharification of pre-treated sunflower stalks, Biomass Bioenerg., 2002, 23, 237-243.

[37] Ortiz-Zamora O., Cortes-Garcia R., Ramirez-Lepe M., Gomez-Rodriquez J., Aguilar-Uscanga M.G., isolation and selection of ethanol-resistant and osmotolerant yeasts from regional agricultural sources in Mexico, J. Food Process Eng., 2009, 32(5), 775-786.

[38] Gera R., Dhamija S.S., Gera T., Singh D., Intergeneric ethanol producing hybrids of thermotolerant Kluyveromyces and non-thermotolerant Saccharomyces cerevisiae, Biotechnol Lett., 1997, 19(2), 189-194.

[39] Ge X.M., Zhang L., Bai F.W., Impacts of temperature, pH, davalent cations, sugars and ethanol on the flocculating of SPSC01, Enzyme Microb. Tech., 2006, 39, 783-787.

[40] Kiran Sree N., Sridhar M., Suresh K., Banat I.M., Venkateswar Rao L., Isolation of thermotolerant, osmotolerant, flocculating
Saccharomyces cerevisiae for ethanol production. Bioresour Technol., 2000, 72(1), 43-46.

[41] Konar E.M., Harde S.M., Kagliwal L.D., Singhal R.S., Value-added bioethanol from spent ginger obtained after oleoresin extraction, Ind. Crops Prod., 2013, 42, 299-307.

[42] Fakruddin M.M., Abdul-Quayum M., Ahmed M., Choudhury N., Analysis of key factors affecting ethanol production by Saccharomyces cerevisiae IFST-072011, Biotechnol., 11, 248-252.

[43] Joshi J., Enhanced production of ethanol from red potatoes grown in hilly regions of Nepal using various nitrogen sources, Int. J. Appl. Sci. Biotechnol., 2014, 2(1), 41-44.

Supplemental Material: The online version of this article (DOI: 10.1515/bioeth-2016-0011) offers supplementary material.