



Concise review on ethanol production from food waste: development and sustainability

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Abstract

The development of sustainable bioethanol fuel production from food waste has increasingly become an attractive topic. Food waste is recognized as the most available and costless feedstock. Therefore, ethanol production has been adopted as cost-efficient and an ecological way for FW disposal. This paper reviewed the microorganisms utilized for ethanol fermentation, the effect of enzymatic hydrolysis on ethanol concentration, optimization of accurate process parameters, and recycling of huge volumes of stillage for ethanol production towards reducing any incurred environmental burdens and minimizing the cost. The statistical tools which may enhance the process efficiency had been presented. Also, the perspective and the future development were introduced. All these aimed to fully utilize the food waste and also reduce the cost for side-product in this process; proper operation conditions and the control methods for stillage recycling were considered as the methods to improve ethanol fermentation from food waste.

Keywords Food waste · Ethanol fermentation · Microorganisms · Fermentation optimization parameters · Stillage recycling technology · Metabolic regulation

Introduction

Studies on ethanol production from waste have been accelerating recently due to both environmental and economical aspects (Man et al. 2010). Ethanol has been recognized as the cleaner biofuel because their net emission for CO₂ during combustion was zero (Vohra et al. 2014). Thus, it was found to have huge environmental benefits when utilized as a part of fuel mixture to diminish the emission of hazard gases in vehicles (Kim and Dale 2004). Although ethanol fuel has lower

energy density than methane, H₂ and energy equivalent was 68% lower than that of fossil fuel (Vohra et al. 2014); its liquidity and superior production rate makes it attractive as a transport fuel. For instance, it was observed that the generation and combustion of ethanol decreases GHG emanations by 12% as compared with gasoline (Hill et al. 2006). Therefore, it has been used in the concentration range of 10–85% (v/v) for the gasoline mixture (Vohra et al. 2014). Ethanol manufactured by fermentation occupied by over 90% in the ethanol industry (Zi et al. 2010). However, great amount of ethanol production has been carried out from food-base materials, including sugarcane and corn, which makes bioethanol fuel more costly than fossil fuels (Karmee 2016).

Food waste is considered as the largest part of the municipal solid waste, which contributes by around 9.6% of global CO₂ emissions (Kiran et al. 2014a). Food waste is a part of organic matter that increases due to population and industrialization growth, and is expected to expand in the next 25 years particularly in Asian countries (Chen et al. 2017). Population census may increase to 8.5 billion by 2030 (Seo and Jin 2016). Food waste is a complex biomass containing starchy, fatty, and cellulose materials. However, household garbage and restaurant food waste contain high moisture, organic pollutants, and salinity (Meng et al. 2015). This would cause catastrophic

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effects on environmental and human health. Hence, adequate food waste management system takes place to ensure its ecological and sustainable disposal (Shen et al. 2013). Literature depicts several conventional methods of food waste treatment, including biological and thermal systems, which may cause surface and ground water pollution, greenhouse gas emission, and insect breeding (Goud and Mohan 2011). For example, the greenhouse gas produced in the landfill may reach 1576 kg CO₂e/wet t food waste (Ebner et al. 2014). In contrast, anaerobic digestion and fermentation has been considered to be the most effective methods to achieve renewable energy from food waste as well as the treatment (Pham et al. 2015). Hence, ethanol fermentation from food waste is a plausible alternative for management and disposal of this waste. Although food waste has attractive characteristics, such as being easily converted to fermentable sugars in an environmental friendly way with a low pH, there are some challenges, such as its heterogeneous composition and elevated moisture content, which may vary depending on the resources (Pham et al. 2015). Accordingly, the enzyme type and dosage and the fermentable sugar concentration may vary (Halimatun et al. 2015). Furthermore, the adopted optimal fermentation condition for microorganism to ferment the recovery sugars can contribute to an impediment of the development of an efficient ethanol produced particularly when simultaneous saccharification and fermentation (SSF) was applied (Vohra et al. 2014). Consequently, advanced models for optimization ethanol process conditions via cost-effective approach should be developed. In addition, the effluent and solid residue during ethanol fermentation process needs further treatment before disposed to the environment. Very valuable approach for solving problems related to liquid and solid residual is through the application of integrated systems on ethanol production.

In order to overcome the drawbacks associated with ethanol production from food waste, some studies have been carried out, including optimization of ethanol fermentation process parameters and the utilization of pollutants during the fermentation. This study reviews recent literature data for ethanol production from food waste (including optimum factors influencing ethanol fermentation and stillage recycling technology).

Microorganism utilized for ethanol fermentation from food waste

The potential converting of food waste to bioethanol has been studied over the years. A certain part, such as microorganisms used for ethanol fermentation and the need of enzymatic hydrolysis has been discussed here. In fact, ethanol production from food waste could be performed in three steps: (1) hydrolysis (saccharification) converts the raw material to glucose, (2) fermentations to convert glucose to ethanol and carbone

dioxides, and (3) ethanol separation and purification by distillation. Commonly, ethanol fermentation could be accomplished either by yeast or *Zymomonas mobilis* bacteria (Ma et al. 2008).

The utilization of yeast

Yeast is a fungal cells used for fermentation purposes. Yeast has been adopted for food waste fermentation process. Suwannarat and Ritchie (2015) mentioned that the anaerobic digestion of food waste using yeast could remove 30–50% of its COD and BOD respectively. Yeast is capable to utilize sugars, *Saccharomyces cerevisiae* is efficient in converting glucose to ethanol (Chen 2011); it can also eliminate 50% proteins and lipids. Therefore, *S. cerevisiae* is utilized for stable ethanol fermentation. However, it is imperative that yeasts do not have the full scope of amylolytic enzymes (α -amylase, β -amylase, and glucoamylase) required to completely break down starches to glucose. Yeast has just two genes for amylases, YIL099W (SGA1) and YIR019C (FLO11, MUC1, and STA4). Both of them are α -glucoamylases. Accordingly, starch was mobilized after very long incubation periods (> 20 days), without use of accessory enzymes. Starchy-based substrate, such as food waste has low competence conversion. Therefore, starch conversion can be improved using enzymatic hydrolysis, in which enzyme is used to accelerate the starch hydrolysis for glucose production (Zhang et al. 2016). Ethanol production by *S. cerevisiae* can be achieved in either simultaneous saccharification and fermentation (SSF) or separated hydrolysis and fermentation (SHF) process. In SSF, both saccharification and fermentation can be carried out in one reactor, which is more cost-efficient than SHF. Despite that, the optimum factors affecting saccharification and fermentation are varied (Vohra et al. 2014). Ethanol fermentation process by yeast seems to be redox reaction (biochemical process in cells), in which oxidation-reduction potential (ORP) control is needed in a proper range (Ma et al. 2016a).

The utilization of bacteria

There are some techniques used for bacterial contamination prohibit during ethanol production from food waste, including sterilization, acidic, anaerobic condition, and the inoculation of lactic acid bacteria (LAB) (Zhang et al. 2012). The incubation of LAB converts food waste to lactic acid, which can be oxidized to pyruvate; consequently, ethanol fermentation can be implemented (Ma et al. 2014). Open fermentation process with acid as a method to acting as bacteria inhibition was adopted. In order to accustom to the acid condition, the acid-tolerant *Z. mobilis* can be employed for ethanol production from food waste. *Z. mobilis* has a number of desired features for its special Entner-Doudoroff pathway, which makes it suitable for metabolic engineering (He et al. 2014). Although

some published studies concerning ethanol production from kitchen garbage by *Z. mobilis* uses SSF process, *Z. mobilis* was pointed out to only convert a simple sugar such as glucose. Therefore, there is significant need of adding enzymes when using food waste as feedstock for ethanol production. According to the study by Ma et al. (2016) on the use of an acid-tolerant mutant of *Z. mobilis* (ZMA7-2) in ethanol fermentation process under non-sterilized condition (open fermentation), such process could achieve a higher ethanol production (99.78 g/L) with shorter time (44 h), which was beneficial for an industrial-scale application. Other microorganisms could be used for ethanol production from food waste. Considering the performance of ethanol fermentation using some kinds of microorganism, efficiency enhancement has been done via culturing and co-culturing of these microorganisms.

Application of flocculation yeast

In efficient process, the fermented broth should contain 8–14% (v/v) of ethanol. Greater than this concentration may cause yeast inhibition and reduce their activity. Therefore, to improve yeast activity and enhance fermentation productivity, the innovation techniques should be applied (Vohra et al. 2014). Hence, the selection of new yeast strains, which has further adapted to stress conditions of ethanol fermentation has been investigated. Flocculation of yeast is an effective technology, which provides cell recycles by isolating yeast cells from the culture broth after in situ settling of cells in the bioreactor (Ge and Bai 2006; Ma et al. 2009). The application of flocculation yeast may reduce the by-products accumulation during stillage recycling in ethanol fermentation (Zi et al. 2013). In this regard, the flocculating yeast *S. cerevisiae* strain KF-7 was applied for continuous ethanol fermentation, and maximum ethanol produced was 24 g/l/h at a high dilution rate of 0.8/h (Tang et al. 2008). Another study conducted by Ma et al. (2009), on flocculent *S. cerevisiae* strain KRM-1 and its application on kitchen waste ethanol fermentation, revealed that the mutant flocculent yeast was efficient in food waste utilization to bioethanol particularly when batches were repeated. Their findings showed that the mean value of ethanol productivity 8.25 g/l/h was obtained over ten batches of fermentation and as maximum as 10.08 g/l/h in the last batch.

Co-culture of microorganism

Co-culture of microorganism, also known as mixed culture, is the cultured of two or more microorganism together and concurrently exist in the same medium. It has extensively explored to enhanced digestibility of food waste. For example, food waste decomposition in using co-culture of the two bacterial strains (*Bacillus subtilis* (F2) and *Paenibacillus polymyxa* (F8)), was relatively lower than that with four

bacteria strains (*B. subtilis* (F2), *Paenibacillus* (F5), *Bacillus cereus* (F6), and *Pseudomonas* (F7)), which clearly represented that the co-culture system encouraged food waste degradability (Li et al. 2014).

Considering the various possibilities of resource recovery, fermenting of mixture of sugars into bioethanol is one of the key areas to be addressed. Since there is no native microbe capable to convert all sugars present in hydrolysate broth into high concentration ethanol (Lin and Tanaka 2006), thus, co-culture systems can be used for efficient digestion of all sugars in hydrolyzed broth, increasing the volumetric ethanol production, shortening the fermentation time, and minimizing production cost (Chen 2011). For example, co-culture of *S. cerevisiae* and *Enterobacter aerogenes* has increased the ethanol yield from stillage to about 1.76 g Eth/g sugar (Choonut et al. 2015). Since glucose and xylose are the dominant sugars composition found in the hydrolyzed broth, there are some microbes capable to ferment glucose and unable to metabolize xylose, such as *S. cerevisiae*, which is so far used for ethanol production. And others can ferment xylose, such as *Pichia stipitis*. Therefore, application of the co-culture system consists of a *respiratory-deficient mutant S. cerevisiae* and *P. stipitis* to elevate the fermentation performance of hydrolyzed medium containing both sugars have shown promising results (Kordowska-Wiater and Targon'ski 2001). Vast researches showed the application of co-culture system for ethanol production (including co-culture of, immobilized *Z. mobilis* with free cells of *P. stipitis*; ethanologenic *E. coli* strain KO11 with *S. cerevisiae*; *S. cerevisiae* with *Pachysolen tannophilis*; and *Z. mobilis* with *Candida tropicalis*, etc.). Nevertheless, the best finding obtained for fermenting glucose and xylose is via using co-culture of immobilized *Z. mobilis* and *P. stipitis* for ethanol production from different biomass materials including FW (Chen 2011). Co-culture also utilized for pentose and hexose fermentation from lignocellulosic biomass. The success of co-culture system depends mainly on the rate of growth of various microorganisms on the different hydrolyzed medium and the compatibility to the fermentation condition, including temperature and pH (Cardona and Sánchez 2007).

Enzymatic hydrolysis update

Enzymatic hydrolysis is the first stride in majority of bioprocesses for food waste valorization. It is recognized as a perfect pre-treatment approach in ethanol production from food waste (Kiran and Liu 2015). Hydrolysis process yields sugars, including glucose, fructose, xylose, ribose, and galactose. The various kinds of food waste have varied sugars composition, and sugars yield depend mainly on the food waste nature and composition (Halimatun et al. 2015). The ethanol productivity and concentration may vary with respect to

sugars concentration in the hydrolysate broth. However, the selection of perfect enzyme with respect to FW composition can promote the hydrolysis process. Considering several types of food waste utilized in the production of different enzymes, including amylase, protease, cellulase, lipase, and pectinase, which so far have been used for food waste hydrolysis (Kiran et al. 2014b). Enzyme is too costly to be economically viable. Recently, in situ-produced fungal mash from food waste, which is wealthy in different hydrolytic enzymes, has been applied on the ultra-fast hydrolysis of FW (Ma et al. 2017).

Several studies in Table 1 have shown that the use of enzyme in ethanol production from FW could improve its productivity by elevated glucose recovery. Moon et al. (2010) has used a mixture of carbohydrases and amyloglucosidases to produce ethanol from food waste; those two enzymes are mainly hydrolyzed, the starch and cellulose in food waste. Maximum glucose and ethanol yield were in this order 0.46 g/g of dry FW and 29.1 g/L. Furthermore, Uncu and Cekmecelioglu (2011) have generated (32.2 g/L) ethanol in a study of food waste pre-treated with amylases. Also, amyloglucosidase, α -amylase, and protease were together mixed and applied for ethanol production from food waste

(Hong and Yoon 2011). Another study showed that 127 g/l glucose was recovery at 24 h from mixed food waste pre-treated by fungal mash, elevating ethanol concentration to 58 g/l. In summary, the dominant enzyme used in food waste hydrolysis for ethanol production is glucoamylase, also known as amyloglucosidase. The reason is that food waste is rich in carbohydrates and starches, and this enzyme is responsible of breaking off long-chain carbohydrates and starches into sugar that will afterwards convert to ethanol by yeast.

Influencing factors on ethanol fermentation

There are many factors that affect ethanol fermentation from food waste, such as physical and chemical characteristics of organic components and the fermentation environment, including pH, temperature, and culture time. Furthermore, the parameter related to food waste composition, such as moisture contents (S/L ratio). The inoculum size has been detected to have significant effect on ethanol production. Moreover, the ethanol productivity was depending mainly on glucose recovery, which depends on the type and optimal dosage of enzyme

Table 1 Various kinds of enzyme applied for food waste hydrolyzed

| Enzyme | Dosage | Benefits | Resource | Reference |
|---|------------------------------|---|---|-------------------------------|
| Amyloglucosidases | 2.0 AGU/g | Hydrolysis of starch, elevated G. C. to 52 g/l and R. S to 61 g/l | Novozymes Korea, Seoul, Korea | Moon et al. (2010) |
| Carbohydrases | 20.0 FBGU/g | Cell-wall degrading enzyme, elevated G. C. to 44 g/l and R. S to 63 g/l | Novozymes Korea, Seoul, Korea | Moon et al. (2010) |
| α -Amylases (A6211-1MU) | 120 U/g | Starch liquefaction | SIGMA–Aldrich | Uncu and Cekmecelioglu (2011) |
| Fungal mash | 16 g/l | Rich in various hydrolytic enzyme | In situ produced from FW | Ma et al. 2017 |
| Nagase N-40 glucoamylase | 170 mg protein/kg WW | Glucose recovery increased to 85.5%, elevated ethanol to 24 g/l/h | Nagase ChemteX Corporation, Osaka, Japan | Tang et al. 2008 |
| Glucoamylase | 100 U/g | Elevated R.S. to 75 g/l and ethanol conc. To 53.40 g/l | Beijing Dong Hua Qiang Sheng biochemical technology company | Ma et al. 2008 |
| Glucoamylase | 180 mg protein/kg WW | R.S. is 200 g/l and ethanol conc. 99.78 g/l | Glucoteme, 20,000 U/g; Nagase sangyo, Osaka, Japan | Ma et al. 2016 |
| Protease | 100 U/g | Elevated R.S. to 75 g/l and ethanol conc. to 53.40 g/l | Beijing Dong Hua Qiang Sheng biochemical technology company | Ma et al. 2008 |
| Mixture of cellulase and β -glycosidase | 83 FPU/ml (ratio 5:1 (v/v)) | Increase the microorganism mixing rate and increase ethanol production and productivity | Novozymes A/S (Bagsværd, Denmark) | Matsakas et al 2014 |
| Mixture of carbohydrases and amyloglucosidases | 20.0 FBGU/g & 2.0 AGU/g | Elevated G. C. to 58 g/l and R. S to 71 g/l | Novozymes Korea | Moon et al. (2010) |
| SAN super 240 L (mixture of protease, α -amylase, and amyloglucosidase). | 240 AGU/ml (0.5 ml/100 g FW) | Glucose yield of 600 g/kg | Novozymes, Denmark | Hong and Yoon 2011 |

WW wet waste, G.C glucose concentration, R. S reducing sugar

used for food waste hydrolyzed (Ma et al. 2016). It should be mentioned that pH and temperature were the most important factors affecting ethanol production, as indicated by the vast amount of research published concerning their adaptation and optimization.

Effect of pH on ethanol fermentation

pH affects the shape of proteins. In the case of fermentation, a collection of enzymes is responsible for the metabolic processes that occur. If the pH is increased, it affects the shape of proteins by disrupting the bonds in the protein. With regard to the effect of pH on ethanol fermentation from food waste, initial pH value of FW at a range of 4–5. The effect of different pH on ethanol fermentation process kinetics' was studied at a range of 3.5–6; fermentation efficiency, cell density (OD_{600}), and ethanol concentration were increased when pH rose from (3.5–4) in this order (97.16%, 3.9, and 99.6 g/l), then gradually decreased with pH increased from 5–6 to reach 74.6%, 3.52, and 76.5 g/l) respectively (Ma et al. 2016). Another study showed the effect of pH on ethanol fermentation using saccharified broth with pH of 3.5, 4, and 4.5. Maximum ethanol concentration of about 30 g/l has been obtained at pH 4–4.5 (Tang et al. 2008).

Effect of temperature on ethanol fermentation

Temperature changes have deep effects upon microorganism's activities by influencing an enzyme-catalyzed reaction. Throughout the past years, the studies of decrease overall cost of ethanol production has been carried out by reducing the energy (temperature) consumed, which is considered as the second top cost in ethanol production (Zi et al. 2013). Traditionally, the conversion of carbohydrates to oligosaccharides and glucose was achieved by a thermophilic α -amylase under high temperature (95–105 °C) in the saccharification process and at pH 6.0–6.5. This requires intensive energy that leads to an increment in production cost (Xu et al. 2016). Then, oligosaccharides converted to glucose through addition of glucoamylase from a fungus for further hydrolysis under low temperature (60–65 °C) at pH 4.0–4.5. The reduction of temperature demand during hydrolysis process can be achieved if the starch hydrolysis is accomplished within temperature below its gelatinization temperature (Robertson et al. 2006). However, food waste has a variety of components, such as proteins and cellulose, which need other kind of enzymes besides amylase to hydrolysis it. In addition, the saccharification temperature influences growth of energetic bacteria existing in the non-sterilized saccharified broth. Therefore, the use of two genes of glucoamylase (Glucochimu#20000 and Nagase N-40) for glucose recovery have been investigated under various temperatures (50–60 °C) pointing out that glucose yield efficiency increased from 75.8

to 85.5%, when the saccharification temperature increased from 50 to 60 °C. In contrast with Glucochimu #20000, in which glucose yield decreased from 72.0 to 25.4% when temperature increased from 50–60 °C, demonstrating that the Nagase N-40 glucoamylase was preferable at saccharification and ethanol yield than Glucochimu #20000 at 60 °C (Tang et al. 2008). While, the fermentation process takes place at temperature between 25 and 30 °C depending on the feedstock characteristics and composition (Vohra et al. 2014), fermentation temperature for food waste to ethanol is in the range of 30–37 °C, which is because the microorganism becomes more active when warm and dies at high temperature.

Effect of inoculum size on ethanol fermentation

Another contributing factor on ethanol production is the inoculum size. However, the inoculum size has direct effect on cell density as well as ethanol fermentation. Low inoculum size causes less growth of cell and low ethanol production, when high inoculum size causes over growth of cells, which may lead to their contest on substrate (Ma et al. 2008; Ma et al. 2016).

Effect of moisture content on ethanol fermentation

The moisture content of food waste has a significant effect on microorganism growth and activities. The superior solid-liquid ratio would yield high ethanol concentration, which would affect microorganism activities (Ma et al. 2008). When the (S/L) ratio was low, it would not negatively affect microorganism, but high energy is needed for distillation process, which would enhance the ethanol production cost. The high proportion meant a high concentration of the substrate, which might generate high osmotic pressure that is favored by-product synthesis, such as sucrosebitol and levan (Oursson and Rohmer 1992).

Effect of the incubation time on ethanol fermentation

The incubation time has great effect on ethanol fermentation efficiency due to a cumulative of other factors. Prolong of fermentation time may increase ethanol production cost by increasing the energy consumption. Optimal time can enhance ethanol productivity by mitigation of side-product accumulation, such as organic acids and glycerol, which may inhibit yeast activity (Ma et al. 2016a).

Optimization of significant factors in ethanol production

Optimization of various process factors affecting ethanol production is a complex process with a number of interactive

controlling parameters. At industrial level, even a small improvement in the process gives a better yield, which may be beneficial commercially, making the process of optimization a key area of research in the field of industrial biotechnology. The major challenges for marketing the food waste to bioethanol production technology are energy efficiency and cost benefits (Sen et al. 2016). An increased accumulation of non-degradable metabolic intermediates provides an indication of possible process imbalances. The best way to eliminate the cost of ethanol production is through the proper choice of substrate to be followed by optimization of process parameters (Ma et al. 2008). For a particular process variable, the optimum value is determined considering the remaining process parameters are fixed at optimum condition. However, the selection of the process operating parameters depends on the type and composition of the substrate.

Optimization of parameters and the chemical nutrient for ethanol fermentation

Food waste contains sufficient amount of chemical nutrients, which is required for microorganism to produce ethanol. Hence, chemical additive to the fermentation system, including $(\text{NH}_4)_2\text{SO}_4$ and KH_2PO_4 have showed less impact on ethanol production and concentration (Ma et al. 2008; Tang et al. 2008; Thongdumy et al. 2014). Nevertheless, calcium ions can increase flocculation rate of *S. cerevisiae* KRM-1 by more than 85% in kitchen waste ethanol fermentation (Ma et al. 2009).

As presented in Table 2, optimal pH for most substrates with a range of 4–6 indicated that the activities of microorganism to digest organic matter have a limited acidity range condition. It should be mentioned that, when the pH of fermentation media is higher than the optimal, yeast produces acid instead of ethanol (Tahir et al. 2010). Investigations explored that most types of food waste have pH around 4–5, which was viable for ethanol fermentation (Ma et al. 2008; Tang et al. 2008; Man et al. 2010; Ma et al. 2016). Majority of the optimal saccharification temperature for various substrates was in the range 50–60 °C, which demonstrated that the suitable temperature for enzyme to convert substrate contents to glucose takes place within this range. For example, the optimal temperature for saccharification using SAN 240 L enzymes was 50 °C (Hong and Yoon 2011). While the optimal temperature using glucoamylase enzymes was 60 °C (Tang et al. 2008), the optimal temperature for fermentation process using FW was found as 30–35 °C (Tang et al. 2008; Ma et al. 2008; Ma et al. 2016). Sweet sorghum juice fermentation temperature was 27.7 °C, while maximum ethanol yields from Korean food waste leachate was obtained under optimum temperature of 38 °C (Man et al. 2010).

In an effort to enhance the process of ethanol conversion from food waste, there are other factors which need to be

optimized, including optimal enzyme for hydrolysis, glucose concentration, inoculum size, solid to liquid ratio, and fermentation time. The most significant factor which may affect ethanol productivity is an optimal enzyme used for saccharification process. Therefore, evolutionary engineering approaches have been used to select the proper enzyme for ethanol production. Accordingly, glucoamylase and protease were revealed to have significant effect on ethanol fermentation. And the optimal dosage for both enzymes was 100 U/g (Ma et al. 2008). Another large issue which may inhibit ethanol production by slowing down the hydrolysis and yielding low level of usable hydrolysis product is the excess glucose present in the system. However, a glucose concentration higher than 200 g/l may slow cell growth and greatly affect cell viability, which will have negative influence on ethanol fermentation performance (Ma et al. 2016). Initial inoculum size of around 10% (v/v) was the optimal for most FW types. While the optimal inoculum size for sweet sorghum juice was in the range of 5–7.5%, the lowest inoculum size was 3% for sugar cane molasses. Optimal ratio of S/L which showed relatively elevated ethanol yield from FW was 1:0.5 (Ma et al. 2008). Optimal culture time for ethanol production from food waste is 40 h, which is more viable for an economical aspect (Ma et al. 2008), in comparison to the high optimal incubation time obtained for ethanol fermentation from fresh jackfruit seeds, which was 124.5 h (Chongkhong et al. 2012).

Based on Table 2, keeping in consideration the different feedstock used for ethanol production, the studied showed different ethanol concentrations under optimal conditions. For instance, sweet potato showed about 176 g/l; most food waste showed more than 30 g/l, sugar cane around 60 g/l, when the lowest was wheat straw 16.4 g/l. The elevated concentration from sweet potato was due to the application of co-culture technology. The disadvantage of sweet potato was the longer fermentation time (72 h) as compared with that of food waste (around 40–48 h).

Common statistical models used

Statistical models can be used to enhance the production of special substrate by optimization of operation factors. Hence, the use of statistical instruments can help researchers to improve products and enhance the process efficiency. The most common statistical method, which has been used by various researchers in the biotechnology field is the response surface methodology (RSM) (Walia et al. 2014). RSM is defined as a set of mathematical and statistical method-based experiential (Wang et al. 2011). The main function of RSM is to estimate the effect of various factors responsible for the ethanol production, and then optimize them (Dash et al. 2017). Therefore, most ethanol fermentation parameters have been optimized using RSM associated with a sequential statistical model. Recently, the ethanol fermentation parameters (including pH,

Table 2 Optimum parameters required for maximum ethanol production from different waste types in comparison to food waste

| Waste type | Condition | Microorganism | Optimum parameters | | | | | | G.C g/l | E.C g/l | E.Y % | Reference |
|----------------------------|------------------|-------------------------------|--------------------|------------|------------|---------------|--------------|---------|------------|------------|----------|-------------------------------|
| | | | pH | S. T °C | F. T °C | F. t hours | I. size % | S/L | | | | |
| Food waste | NR | <i>S. cerevisiae</i> | 4.5 | 60 | 30 | 48 | 8.9 | NR | NR | 32.2 | NR | Uncu and Cekmecelioglu (2011) |
| Kitchen garbage | Sterilized | <i>Z. mobilis</i> 10225 | 5 | 60 | 35 | 40 | 10 | 01:00.5 | NR | 53.4 | NR | Ma et al. (2008) |
| Kitchen garbage | LAB | Yeast strain KF-7 | 4 | 60 | 30 | 42 | NR | NR | 74 | 29.9 | NR | Tang et al. (2008) |
| Food waste | Non-sterilized | ZMA7-2 | 4 | 50 | 30 | 44 | 10 | NR | 200 | 99.78 | NR | Ma et al. (2016) |
| Korean food waste leachate | NR | <i>S. cerevisiae</i> | 5.45 | NR | 38 | NR | NR | NR | NR | 24.17 | NR | Man et al. (2010) |
| Sweet sorghum juice | NR | SCS NRRL Y-2034 | 5.4 | NR | 27.7 | NR | 5 | NR | NR | NR | 9.3 | Wang et al. (2011) |
| Sweet sorghum juice | NR | SCS NRRL Y-2034 | 5.4 | NR | 30 | NR | 7.5 | NR | NR | NR | 8.83 | Phutela and Kaur (2014) |
| Coffee pulp waste | Sterilized | <i>Pichia anomala</i> M4 | 4.5 | NR | 30 | NR | NR | NR | NR | NR | 4.07 | Hamadi et al. (2014) |
| Ziziphus mauritiana | NR | <i>S. cerevisiae</i> NA33 | 6 | NR | 30 | NR | NR | NR | NR | 63 | NR | Togarepi (2012) |
| Corn | NR | <i>S. cerevisiae</i> MTCC4043 | 5.8 | NR | 31 | NR | NR | NR | NR | 74.6 | NR | Walia et al. (2014) |
| Sago starch | NR | <i>Z. mobilis</i> ZM4 | 5.02 | NR | 36.74 | 17 | NR | NR | NR | 70.68 | 97.08 | Bvv et al. (2003) |
| Sweet potato | NR | SC and <i>Pichia</i> sp. | 5 | NR | 30 | 72 | 10 | NR | NR | 127 | NR | Dash et al. (2017) |
| Sugar cane molasses | NR | <i>Z. mobilis</i> | 5.13 | NR | 31 | 44 | NR | NR | NR | 58.4 | NR | Maiti et al. (2011) |
| Sugar cane molasses | NR | <i>S. cerevisiae</i> BIO-07 | 4.5 | NR | 30 | NR | 3 | NR | NR | 76.8 | NR | Tahir et al. (2010) |
| Fresh jackfruit seeds | NR | Loog-pang Kao Mhark | 5.2 | NR | 32.2 | 124.5 | NR | NR | NR | NR | 11.5 | Chongkhong et al. (2012) |
| Wheat straw | Microwave alkali | <i>S. cerevisiae</i> | 5.5 | NR | 30 | NR | 3.3 | NR | NR | 16.4 | NR | Singh and Bishnoi (2013) |

Pichia anomala M4 is yeast strain

SC *Saccharomyces cerevisiae*, LAB lactic acid bacteria, ZMA7-2 acid-tolerant mutant of *Zymomonas mobilis*, S. T Saccharification temperature, F. T fermentation temperature, Ft fermentation time, G. C glucose concentration, E. C ethanol concentration, SCS *Saccharomyces cerevisiae* strain

incubation temperature, inoculum size, and fermentation time) have been optimized using one variable at a time (OVAT) methodology followed by Box-Behnken design of RSM to detect the most significant variable for the maximum ethanol fermentation and the interaction among them (Dash et al. 2017). Also, Plackett-Burman design (PBD) can be used to screen out the factors affecting ethanol production (Ma et al. 2008; Singh and Bishnoi 2013). In addition, the central composite design (CCD) can be used to study the optimum factor levels in ethanol production (Chongkhong et al. 2012). For example, RSM based on the 2³ factorial CCD was used for optimization of the parameters affecting food waste enzymatic saccharification and ethanol fermentation. It showed that the optimal factors of saccharification pH, enzyme hydrolysis temperature, enzyme concentration, fermentation pH, fermentation temperature, and fermentation time were in this order: 5.20, 46.3 °C, 0.16% (v/v), 6.85, 35.3 °C, 14 h. The model expected that maximum reducing sugar concentration and

ethanol fermentation under these optimum conditions were 117 and 57.6 g/l respectively (Jungkon et al. 2008). Most popular statistical methods used in optimization of ethanol fermentation parameters from various substrates are reflected in Table 3.

More values from stillage of ethanol fermentation

Ethanol fermentation involves many challenges, owing to the necessity of feedstock selection as well as pollution monitoring (Ma et al. 2016a). However, the major pollutant during ethanol fermentation of food waste was the dispose of huge amounts of wastewater, which has special characteristics of saline and oil contents. There are concrete steps used to produce stillage (the liquid part of broth) by distilling out the ethanol after fermentation. Then, the broth was centrifuged to get liquid part and solid part, liquid part was named stillage or vinasse (Fuess and Garcia 2015). Stillage eluted from

Table 3 Most common statistical methods using for ethanol production optimization

| Substrate | Factors optimized | Statistical methods | Reference |
|------------------------------|---|-----------------------|-------------------------------|
| Food waste | PH, T,S/L, IS, It | RSM | Uncu and Cekmecelioglu (2011) |
| Kitchen garbage | PH, T,S/L, IS, It, and 7 enzyme | PBD and single factor | Ma et al. (2008) |
| Stalk juice of sweet sorghum | (NH ₄) ₂ SO ₄ , and PSR | BBD-based RSM | Mei et al. (2009) |
| Sweet sorghum juice | T, PH, IS | BBD-based RSM | Wang et al. (2011) |
| Corn | T, pH, and sub. concentration | CCD-based RSM | Walia et al. (2014) |
| Korean food waste leachate | T, PH, RSC | CCD-based RSM | Man et al. (2010) |
| Wheat straw | T, PH, IS, TRS | PBD and BBD | Singh and Bishnoi (2013) |
| Sweet potato root flour | T, PH, It | OVAT and RSM | Dash et al. (2017) |
| Fresh jackfruit seeds | T, PH, It | CCD of RSM | Chongkhong et al. (2012) |

PSR particles stuffing rate, RSC reducing sugar concentration, TRS total reducing sugar

ethanol production is acidic with pH 3.8 to 4.7, COD 90 g/L, suspended solids (20–30 g/L), and total nitrogen of 6 g/l (Choonut et al. 2015). The discharge of this wastewater without appropriate treatment would cause serious eutrophication of water bodies. Therefore, it is a critical issue to find an environmentally and economically sound way to treat such kind of wastewater during the application of ethanol fermentation of food waste. It has been claimed by Ma et al. (2008) that 1 ton of ethanol fermented from food waste could produce about 10 tons of wastewater. Considering the partial utilization of organic contents in food waste, there are more values which can gain from stillage.

Stillage as feedstock

Stillage can be used as potential feedstock for bio-resources recovery; this can lead to an integrated approach addressing sustainability. Stillage is a broth rich in reducing sugar (such as fructose and glucose), in addition to volatile fatty acids (Choonut et al. 2015). In this regard, stillage eluted of ethanol fermentation from different kinds of food waste was reused either as substrate or as process water for ethanol production. The mixture of saccharified residue and stillage from FW ethanol fermentation was used as substrate for methane production, gaining 65.1 l biogas with 50% methane (Tang et al. 2008). Pineapple-ethanol stillage can play as raw material for ethanol production with the maximum yield of 1.76 g ethanol/g sugar after 72 h fermentation using co-culture of *E. aerogenes* and *S. cerevisiae* (Choonut et al. 2015). In addition, stillage from family garbage has been utilized as substrate for methane production; a total of 85% of the energy was obtained from garbage as ethanol and methane (Koike et al. 2009). Reutilization of the stillage technology generates revenue of the biofuel besides wastewater treatment.

Stillage recycles fermentation in food waste (pros and cons)

Stillage treatment represents high issue especially when applying the multi-evaporation method; this consumed energy then increased the production cost (Zi et al. 2013). Recent research affirmed that advanced oxidation technology and other biochemical treatment methods were used to treat such kind of wastewater (Blonskaja et al. 2003; Chaudhari et al. 2005). The main drawback to these methods was the high cost. Another important point is that organic contents of wastewater eluted from ethanol fermentation make it useful to recycle for ethanol production (Ma et al. 2008). As a result, stillage was used instead of tap water for ethanol production. Ethanol yield was 50 g/l, which is higher than those produced with tap water (Ma et al. 2008). However, the challenge associated with stillage recycling was by-product accumulation, which may reduce ethanol productivity. A study carried out by Ma et al. (2016a) revealed that the recycling of stillage increased ethanol concentration from 25 g/l in the first batch to 35 g/l in the fifth batch, and then it reduced in the subsequent batches. On the other hand, it increased fermentation time, which may lead to by-product accumulation, such as salt and organic acids. Therefore, the growth and metabolism of yeast demonstrated inhibitory effects due to viscosity increment, resulting in reduced ethanol productivity. Nevertheless, flocculating yeast could perform continuous ethanol fermentation using stillage recycling technology with a little by-product accumulation (Zi et al. 2013).

Inhibition during stillage recirculation

One of attractive technology for treatment and utilization of food waste and wastewater produced in large quantities during ethanol fermentation process is the stillage recycling

fermentation system. It is recognized as the key for fully utilize substrate resources and enhance ethanol yield with zero wastewater discharge and low energy consumption. In addition, it reduces cost of tap water consumed in large quantities for ethanol production (Tao et al. 2005). However, the accumulation of the by-product has set drawbacks for the technology, and by increasing recycling time, it would inhibit ethanol yield (Ma et al. 2016a). Ohashi et al. (1998) established an integrated system of ethanol production with zero wastewater, using cell retention and distillation system.

Organic acids accumulation inhibition

There are various organic acids accumulated during ethanol fermentation, such as lactic, acetic, propionic, butyrate, and pentanoic acids, etc. (Zi et al. 2013; Castro and Gil 2015; Ma et al. 2016a). Although the boiling point of all these organic acids was recorded as high as 100 °C, nonetheless, their flash point was not high. The study carried out by Zi et al. (2013) showed that there is no harm of organic acid on yeast growth detected during stillage recycling because of their low concentration. Ma's study revealed that the concentration of these organic acids except lactic acid was constant. Therefore, fermentation time expansion could not cause their accumulation. The boiling point of lactic acid is 125 °C, which retards its evaporation during distillation. This evidence is supported that lactic acid would contribute stillage in the following batch of ethanol fermentation. Thus, lactic acid concentration would increase with stillage recycling times, which may reach 120 g/l during fifth time stillage recycling for ethanol fermentation (Ma et al. 2016a). Further increase in recycling time would have negative effect on ethanol fermentation. Evidence indicated that accumulation of lactic acid prolonged fermentation time from 16 to 40 h, which cause toxicity on microorganism that result in ethanol fermentation inhibition. Furthermore, the recycling fermentation system has been applied on a pilot-plant scale. The results showed that the significant inhibitor of ethanol production was accumulation of acetic acid and solids, particularly when recycle continuous (Castro and Gil 2015). Graves et al. (2006) and Liu et al. (2015) claimed that the massive increase of fermentation time in the sixth and seventh times may also express a particular relationship with lactic acid accumulation. Another study showed significant harm affecting yeast growth was due to propionic acid concentration, which produced during sterilization hydrolyzed and distillation (Zi et al. 2013).

Sodium chloride accumulation inhibition

With regard to sodium chloride, salt is naturally present in food waste. However, NaCl content more than 4% w/v can inhibit sugar fermentation by *S. cerevisiae* and cell growth. Therefore, ethanol productivity decreased (Moon et al.

2010). The increase of stillage reflux time has caused accumulation of sodium chloride, which might extend the fermentation time from 16 to 32 h. Evidence from Ma et al. 2016a showed that Na⁺ might produce toxicity to microorganism and negatively influence ethanol fermentation. The author claimed that lactic acid has more influence on stillage reflux ethanol fermentation than sodium chloride.

Osmotic stress inhibition

Osmotic stress happens when the concentration of side-product is elevated within the ethanol fermentation system, which leads to significant osmotic pressure between the inner of the yeast cell and the fermented broth. Consequently, the cell should exert large amounts of energy to conserve a homeostatic balance. Osmotic inhibition is well demonstrated by glycerol inhibition. Glycerol has inhibited effects on the enzymatic hydrolysis, which have been recorded with 2 wt% glycerol in a sugarcane bagasse hydrolysis broth (Zhang et al. 2015). In food waste fermentation case, glycerol accumulation may be caused by the increased repetitive number of stillage recycling. Persistent increment of an osmotic pressure is due to glycerol accumulation. Therefore, yeast cells synthesized abundance of glycerol to resist osmotic stress (Ma et al. 2016a). In the first batches of ethanol fermentation, glycerol contributed in cell growth and by increasing recycling in supplying cell with osmotic stress to protect cells. Inhibition occurs when cell production reduced with an increase in ethanol productivity. This phenomenon may occur at high osmotic stress concentration (Moawad 2012).

Mitigation of inhibition factors during stillage recycling

Most of by-products accumulated within the fermentation system during stillage recirculation is due to feedstock nature, yeast metabolism, and chemical reactions (Zi et al. 2013). The hazardous side-products produced during recycling may prevent the growth and metabolism of yeast (Wei et al. 2015). The application of flocculating yeast strain may mitigate the by-product accumulated in the stillage, where yeast thermal lysis has been prevented and many side reactions would thus be eradicated making potential stillage recycling many times (Zi et al. 2013).

It should be mentioned that lactic acid (LA) has been reported to be the main inhibitor for ethanol production from food waste, particularly when stillage recycling many times. The special characteristics of LA, such as its highly boiling point could make it piled up within the fermentation system (Ma et al. 2016a). There are other by-products due to stillage recycling, such as salt and glycerol, which have fewer hazards. In this context, progress has been made in diagnosis and

mitigation of injurious effect of lateral products in ethanol fermentation with stillage recirculation (Ma et al. 2016b). Accordingly, there were two methods used named as regulation technologies. The first one is by-product regulation. In this method, optimum amount of calcium carbonate was added to the fermentation system began from the fifth batch. Therefore, ethanol concentration enhanced and fermentation time was dropped in this order (30.5 g/l and 48 h). Subsequently, for the other two batches, ethanol concentration was increased to 38 g/l and lactic acid was decreased to 25 g/l. Secondly, the metabolic regulation is concerned with the oxidation-reduction value. The redox regulation function is to diminish the stress effect of the environment. In fact, *S. cerevisiae* is the most significant yeast in glycerol production. This microorganism glycerol plays an important role in physiological processes, such as control osmotic stress, manage cytosolic phosphate levels, and to keep the NAD⁺/NADH redox balance (Scanes and Prior 1998). However, the main task of glycerol is maintaining oxidation–reduction balance of yeast cells and hyper-osmotic stress response (Michnick et al. 1997). In this regard, the significant value of ORP influencing the regulation strategies was detected by adding potassium ferricyanide. This began from the fifth batch of ethanol fermentation; it was found that the optimum ORP value (–150 mV to –250), which increase the ethanol concentration to high than 38.5 g/l compared to that before applying the optimum value and the percentage was 21%. In addition, the fermentation time would decrease from 60 to 48 h in the fifth batch (Ma et al. 2016b). Liu et al. (2015) suggested the same value of ORP in his study on the system of self-flocculating yeast starch fermentation, which improved fermentation and increase ethanol concentration.

However, evidence showed that the metabolic regulation has better effect than by-product regulation. While, the accumulation of glycerol obviously showed that these two methods can mitigate the significant impact (Ma et al. 2016b), there are other methods used to mitigate the by-product in the fermentation system, for example, Zhang et al. (2009) applied the bio-flocculation process to treat the stillage, then recycling it in ethanol fermentation. The process consist of three steps: screening, treatment with polyaspartic acid, and filtration, which produced ethanol as the same concentration with that of tap water.

Perspectives

On the light of the conversion of food waste to ethanol fuel is becoming ecologically creditable and gaining rapid interest. Fuel is the crucial need of mankind all over the world, and food waste management is a key problem, which needs bio-remediation. Therefore, development of sustainable bioethanol production from food waste should be gained

through the control of contaminants related to this process (including waste water and solid waste utilization). Development that is unsustainable may lead to negative effect in economic and social trends in addition to environmental repercussions. Considering various technologies used to treat waste water eluted during ethanol distillation, the potential of ethanol-methane coupling fermentation system on food waste valorization with zero effluent and little energy consumption is the best way. This method could offer maximum utilization of organic content in stillage and eliminate the pollutants release to the environment. Also, it could improve the value of the product and energy recovery efficiency. The feasibility of stillage treated via dry methane fermentation has been tested, and 850 ml of biogas was obtained from 1 g of VTS (Koike et al. 2009). The concepts of this technology is to use stillage from ethanol production as substrate for anaerobic digestion process, then the effluent from AD can use for the consequence batch ethanol fermentation. In order to realize a maximum methane production and increase the rate of organic matter utilization, fermentation parameters (such as substrate/inoculum ratio, PH, temperature) should be optimized. Also, studying influence of such parameters on microbial community may facilitate AD process smoothly and successfully. Furthermore, mixture of stillage and AD effluent can use as substrate for the following batch ethanol fermentation. Ke et al. (2014) were investigating the utilization of stillage and AD effluent mixture for cassava ethanol fermentation, according their findings about 97% COD was removed, 300 ml/g COD methane was produced, and ethanol yield was elevated. On the other hand, a solid residue separated from hydrolysis broth can be used as substrate for methane fermentation, supplies energy for fermentation process, thereby, contributes to the sustainable development of ethanol industry from food waste. Furthermore, solid residue can utilize as bio-fertilizer with regard to its good quality. Direct utilization of solid residue as bio-fertilizer have been tested and found that it had good quality in term of heavy metal and NPK (Ma et al. 2017; Dahiya et al. 2018).

The conventional method to separate ethanol is distillation, which is not efficient economically due to the energy consumption. Also, the high temperature may effect on the characteristics of stillage and it is reuse. Currently, there are many technologies, which can use for ethanol separation, such as adsorption, ozonation, gas striping, nanoporous polymer membrane, flash evaporation system, molecular sieve dehydration units, and pervaporation. Among them, the pervaporation using ethanol permeable and water permeable membrane might be the best due to the lower energy consumption and significant increase in separation performance.

However, there are various concentrations of ethanol produced from FW, for example, Uncu and Cekmecelioglu (2011) obtained 32.2 g/l ethanol, when Ma et al. (2016)

obtained 99.78 g/l under the optimal condition. So, the application of vacuum fermentation in ethanol industry can lead to diminish the microorganism inhibition, causing the increase of ethanol production. Add to this, that distillates of ethanol at the fermentation temperature can save the high energy used for distillation. The perception of this technique is to consistently removal of ethanol from the fermentation reactor via coupling fermentation vessel with a vacuum chamber, so that ethanol distillates at temperature 32 °C. Some techniques used for ethanol removal had mentioned in one review, including vacuum fermentation, gas stripping, and ceramic membranes (Cinelli et al. 2015).

Technology control that merit consideration is the applying of dynamic oversight on process parameters during ethanol fermentation (including pH, temperature, and enzyme dosage) and may maintain the glucose concentration in a desired range, resulting in increased ethanol production and concentration, which depend mainly on glucose recovery in contrary with traditional monitoring. In this regard, we should mention that dynamic oversight is based on site measurement with respect to the various kinds of food waste.

Conclusion

The usage of biotechnological approaches in ethanol production from food waste has been increasing recently due to environmental and economical aspects. Researchers got concerned with optimization of process parameters (including physical-chemical factors and other parameters) in order to yield maximum ethanol with minimum production cost. Furthermore, stillage recycling technology has benefits of full utilization of organic content sources in food waste and keeping cost of water used for ethanol fermentation reasonable. Moreover, further investigation should be carried out to resolve the by-product accumulation problem.

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