

Review

Biotechnological Approaches for Production of High Value Compounds from Bread Waste

¹Saima Haroon, ²Anu Vinthan, ³Leonardo Negron, ²Shantanu Das and ¹Aydin Berenjian

¹School of Engineering, Faculty of Science and Engineering, The University of Waikato, Hamilton, New Zealand

²Goodman Fielder Limited, Auckland, New Zealand

³Callaghan Innovation, 69 Gracefield Road, Lower Hutt, New Zealand

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Corresponding Author:

Aydin Berenjian

School of Engineering, Faculty

of Science and Engineering,

The University of Waikato,

Hamilton, New Zealand

Tel: +64 7 858 5119

Email: aydin.berenjian@waikato.ac.nz

Abstract: A growing global population has led to an increasing demand for food processing industries and consequently the generation of large amounts of food waste. This problem has intensified due to slow progress in the development of effective waste treatment and disposal strategies. Food waste such as bread waste is a reservoir of complex carbohydrates, proteins and lipids which have the potential to be reused in fermentation processes. In this regard, bread waste has been used for the production of a variety of bio-products including ethanol, methane, lactic acid, succinic acid, amylase and protease. This review provides an overview of the previous works in the literature on the utilization of bread waste and the different bio processing approaches to produce a higher value products. Recommendations are also provided for areas of future research.

Keywords: Bread Waste, Fermentation, By-Products, Biomass, Nutraceutical Products

Introduction

Food waste is produced throughout the food life cycle and occurs at different stages of production, processing, retailing and consumption (Uçkun Kiran *et al.*, 2014). The amount of food waste has increased in the last 25 years due to population and economic growth. The amount of food waste production in Asian countries was 278 million tonnes in 2005; this figure is expected to increase 1.5 fold by 2025 reaching 416 million tonnes (Uçkun Kiran *et al.*, 2014). In the European Union, food waste generation is anticipated to rise from 89 million tonnes in 2006 to about 126 million tonnes in 2020 (Pham *et al.*, 2015). The major categories of food wastes are meat, fruit, vegetables and bakery products. It is estimated in New Zealand, more than 122,500 tons of food is thrown away each year which is enough to feed almost 263,000 people, for 12 month (SIN, 2015). Food waste is particularly problematic as it has a significantly high organic content (Mekjinda and Ritchie, 2015), which causes severe environmental and subsequent health problems (Fig. 1) (WRT, 2011).

Based on the Food and Agricultural Organization (FAO) report, food waste not only causes huge economic losses but also results in significant damage to natural resources such as climate, water, land and biodiversity (FAO, 2012). Without a proper treatment, one ton of food waste can result in the emission of 4.5 tonnes

(Uçkun Kiran *et al.*, 2015) of organics into landfills (Kosseva, 2009; FAO, 2012). Reducing the amount of food waste, therefore, would have significant financial and environmental benefit.

Bread Waste

Bread is among the major food waste in many countries around the world. It is estimated that 1.2 million tonnes of bread wasted every year globally. Wastage occurs at bakeries, retail outlets with leftovers and consumer households. It is estimated that 407,000 tonnes of bakery waste is produced in the UK every year, which is approximately 15% of the annual UK bread production (Melikoglu and Webb, 2013). This figure is very similar in many countries around the world including New Zealand. Bakery food comprises 10% of all the food waste (20,575 tonnes per annum) (Fig. 2). These include white bread, mixed grain bread, wheat meal bread and bread roll/baguette (Waste MINZ, 2015).

Typically 100 g of bread contains around 50 g carbohydrate (47 g in the form of starch), 9 g protein, 5 g fat, 0.1 g phosphorus, 2.3 g ash and 28.7 g water. Bread has a short shelf life (4-7 days) and various physical and chemical changes occur during its storage. These include major changes in physiochemical characteristics that impact on taste and aroma (Melikoglu and Webb, 2013; Alibardi and Cossu, 2016).

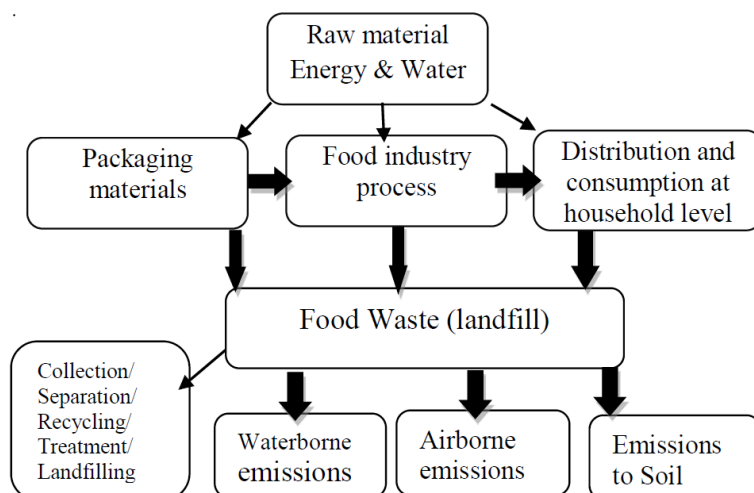


Fig. 1. Food industry waste in the food supply chain, pollutant emissions in air, water and soil (Mekjinda and Ritchie, 2015)

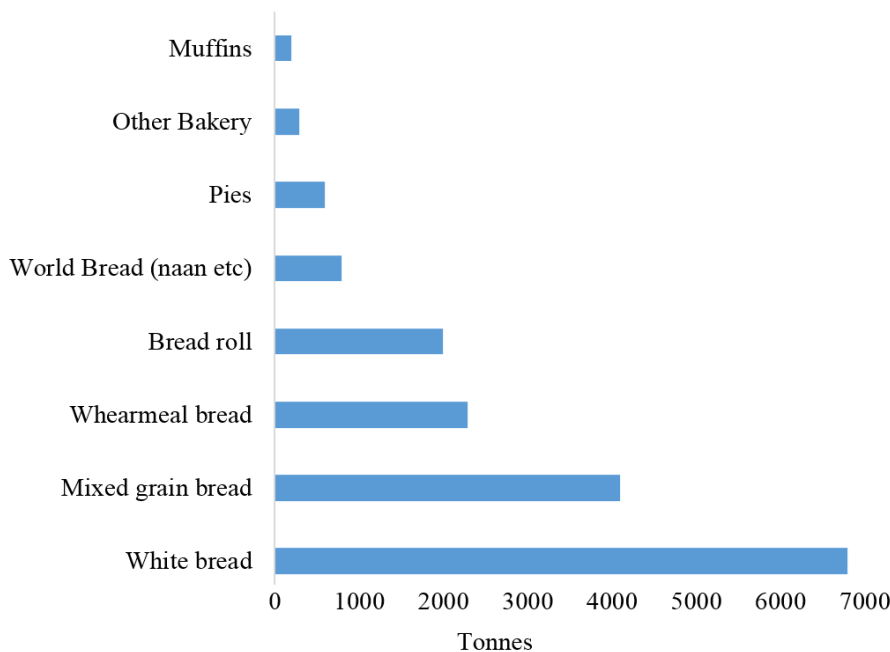


Fig. 2. Bakery waste in tonnes per annum in New Zealand (Waste MINZ, 2015)

Large quantities of bread are discarded due to staling which create economic and environmental problems. Therefore, there is a significant need to develop processes to reduce the associated problems with bread waste formation.

Bioprocess Approaches

Fermentation is a biotechnological process in which complex organic compounds such as carbohydrates and proteins are utilized by fungi or bacteria. During fermentation microorganisms convert nutrients into end metabolites (Leroy and De Vuyst, 2004). Fermentation has been widely used for the production of a wide

variety of compounds. Over the years, fermentation techniques have gained immense importance in waste treatment approaches due to their economic and environmental advantages (Olempska-Beer *et al.*, 2006; Subramaniyam and Vimala, 2012).

Two types of fermentation processes namely Solid State Fermentation (SSF) and submerged or Liquid State Fermentation (LSF) have been dominantly used by researchers. SSF process occurs in low levels of free water and the substrates are solid and insoluble in nature such as grains, wheat bran and vegetables. Due to the low level of free water, this process is more suitable for mycelial fungi than bacteria. SSF process is a simple and

cost effective. It requires lower capital investments and can lead to lower ongoing expenditure. Therefore, SSF can improve economic feasibility of the biotechnological processes by offering waste reduction in design and operation (Kosseva, 2013).

LSF is a controlled process which consists of growing cells in a liquid broth. LSF involves soluble feed stocks since there is more water and less substrates. Moreover, process control and scale up are well established as compared to SSF (Subramaniam and Vimala, 2012). To date, both SSF and LSF approaches have been used for bread waste utilization. The sections below describe the recent and previous work progresses in more details.

Utilization of Bread Waste

The various approaches adopted by different researchers in the area of waste bread utilization are summarized in Table 1. The earliest work on the utilization of bread waste was carried out by Nakano and Yoshida (1977). They used crushed waste bread pieces mixed with molasses, cellulolytic, proteolytic and saccharifying enzymes. The mixture was incubated at 50°C for 75 h to produce a glucose rich syrup which was used as a sugar substitute (Melikoglu and Webb, 2013). Berghofer *et al.* (1995) developed a process for syrup production from waste bread by using hot mash material with malt and enzymatic hydrolysis processes. Daigle *et al.* (1999) also conducted a fermentation on waste bread crumbs for the production of aroma compounds. Fermentation was carried out with 35% white bread crumb and 65% water, using *Geotrichum candidum* ATCC 62217 in Erlenmeyer flasks at 30°C and 300 rpm. Doi *et al.* (2009) have demonstrated the feasibility of bio-hydrogen production from waste bread by using hydrogen-producing bacteria *Megasphaera elsdenii* and *Clostridium species*.

Bioethanol has recently attracted much attention as an alternative resource to fossil fuels. The global market for bioethanol has entered a phase of rapid transitional growth. Many countries around the world are shifting their focus toward renewable sources for power production because of depleting crude oil reserves. Ethanol has high potential as a valuable replacement for gasoline in the transport fuel market. Kumar *et al.* (1998) demonstrated that bakery waste can be utilized to produce ethanol. The bakery waste which included bread, buns, cake and donuts were ground and mixed with water and commercial α -amylase and glucoamylase to hydrolyse the starch. This hydrolysate from starch was then used for ethanol fermentation by using ethanol tolerant *Saccharomyces cerevisiae*. Kawa-Rygielska and Petrzak (2011) also investigated the possibility of using bakery waste as a raw material for ethanol fermentation by *Saccharomyces cerevisiae*.

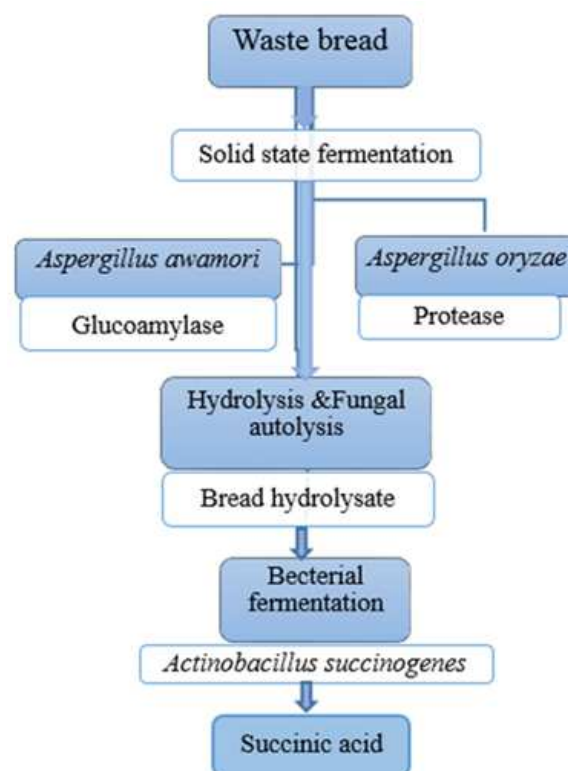


Fig. 3. Process flow diagram of the production of succinic acid from bread waste (Leung *et al.*, 2012)

Table 1. Summary of bio processing research in the utilization of bread waste to value added products (Melikoglu and Webb, 2013)

Publication	End product
Nakano and Yoshida (1977)	Glucose rich syrup
Martin (1984)	Ethanol
Menge <i>et al.</i> (1986)	Lactic acid
Berghofer <i>et al.</i> (1995)	Glucose rich syrup
Oda <i>et al.</i> (1997)	Lactic acid
Kumar <i>et al.</i> (1998)	Ethanol
Meuser (1998)	Ethanol
Daigle <i>et al.</i> (1999)	Aroma compound
Asghar <i>et al.</i> (2002)	α -amylase production
Yahagi <i>et al.</i> (2003)	Starch substitute
Yamashita and Miwa (2003)	Methane
Maeda <i>et al.</i> (2004)	Ethanol
Murase and Yoshino (2005)	Sugar solution
Melikoglu <i>et al.</i> (2013)	Enzyme and ethanol
Doi <i>et al.</i> (2009)	Bio-hydrogen
Leung <i>et al.</i> (2012)	Succinic acid

The yeast for ethanol production was the same as the one used for leavening bread (Kawa-Rygielska and Petrzak, 2011). Utilization of waste bread, for bioethanol production is an innovative solution for recycling bread waste and at the same time reducing its environmental impact in an eco-friendly and profitable way.

Table 2. List of food grade and probiotic microorganisms and their final products

Microorganism	Final metabolite
<i>Lactobacillus acidophilus</i>	Lactic acid, organic acids, hydrogen peroxide, diacetyl, Exo-Polysaccharides (EPS), Lactate (DL) lactones (flavour), Guanosine Triphosphate (GTP) cyclohydrolase enzymes, glucosidases, proteases and amylases (Arendt <i>et al.</i> , 2007)
<i>Lactococcus lactis</i>	γ -Aminobutyric Acid (GABA) α -acetolactate, esters (fruity aroma), diacetyl (buttery flavor), vitamin B ₁₂ , B ₁₁ , Folate (Sybesma <i>et al.</i> , 2003), alanine, low calorie sugar (Hugenholtz and Smid, 2002), glutamate decarboxylase and tagatose production (Bhanwar <i>et al.</i> , 2013).
<i>Lactobacillus brevis</i> + <i>Protease</i>	γ -Aminobutyric Acid (GABA) and peptide (Peñas <i>et al.</i> , 2015). Vitamins D and K
<i>Lactobacillus gasserii</i>	DL-lactic acid, gives texture and nutrition (Fujimura <i>et al.</i> , 2012).
<i>Lactobacillus plantarum</i>	L-alanine (amino acid), lactate
+ <i>Lactococcus lactis</i>	Low-calorie sweetener, such as mannitol, sorbitol, GABA (Coda <i>et al.</i> , 2010).
<i>Lactobacillus reuteri</i>	Cobalamin (vitamin B ₁₂), probiotic oligo and poly-saccharides (Schwab <i>et al.</i> , 2008)
<i>Lactobacillus sanfranciscensis</i>	Exo-Polysaccharide (EPS), lactate, acetate, ethanol and CO ₂ (Ganzle <i>et al.</i> , 1998)
<i>Lactobacillus mindensis</i>	DL-lactate and lysine-D-iso-asparagine (Ehrmann <i>et al.</i> , 2003).
<i>Lactobacillus acidifarinae</i>	DL-lactic acid, acetic acid and ethanol. Arginine is de-aminated to citrulline
<i>Lactobacillus zymae sp.nov</i>	(α -amino acid) (Vancanneyt <i>et al.</i> , 2005)
<i>Lactobacillus plantarum</i>	Lactolin, natural antibiotic. L-lysine, anti-viral amino acid, peptides and antioxidants (Rick Swartzburg, 2009)
<i>Lactobacillus rhamnosus</i>	Vanillin (flavour compound), lactic acid (Ganeden BC 30 Probiotics, 2015)
<i>Lactobacillus bulgaricus</i>	Folate, acetaldehyde, diacetyl, bacteriocins.
<i>Bifidobacterium lactics</i>	Lactulose, inulin, galactooligosaccharides, glucose and variety of proteins.
<i>Bifidobacterium bifidum</i>	Lactate (L+), acetate, vanillin (flavour compound) acetic acid, ethanol and formic acid (Jo Panyko, 2015)
<i>Bifidobacterium infantis</i>	Acetic acid, with lactic and formic acid. Vitamin B, folate thiamine (B ₁), nicotinic acid (a B ₃ derivative) (Ganeden BC 30 Probiotics, 2015).
<i>Bifidobacterium longum</i>	Acetic acid, ethanol, formic acid and succinic acid
<i>Rhizobus oligosporus</i>	Vitamin B ₁₂ and niacin, free amino acids (isoleucine, leucine, lysine, valine, glycine, histidine, tyrosine) and GABA (Knorr, 1998).
<i>Saccharomyces cerevisiae</i> var. <i>boulardii</i>	Aromatic flavours compound, 2- and 3-methylbutanol, methyl-propanoic acid, 3-methylbutanoic acid and 2-phenylethanol (in sourdough bread) (Hansen and Schieberle, 2005). Protease (van der Aa Kühle <i>et al.</i> , 2005)
<i>Aspergillus awamori</i> (fungi)	Multi-enzyme solution (Glucoamylase, phosphatase, xylanase and protease) (Melikoglu <i>et al.</i> , 2013)
<i>Aspergillus niger</i>	Citric acid. β -Glucanase, glucoamylase, glucose-oxidase and lactase (Olempska-Beer <i>et al.</i> , 2006)
<i>Aspergillus oryzae</i>	Glucoamylase, lactase, amylase, maltoryzine and violacetin (Olempska-Beer <i>et al.</i> , 2006)
<i>Rhizopus oryzae</i>	Lactic acid and fumaric acid (Skory, 2004). Aroma, aspartic proteinase, glucose oxidase, lipase and laccase (Olempska-Beer <i>et al.</i> , 2006)
<i>Bacillus coagulans</i> GBI-30, 6086	L-lactic acid
<i>Bacillus subtilis</i> (natto)	Protease, α -amylase, vitamin K, vitamin B ₁₂ , Poly glycolic acid (PGA)
<i>Saccharomyces boulardii</i>	Serine, protease and phosphatase that destroys the endotoxin (Keesidis and Pothoulakis, 2012).
<i>Streptococcus thermophilus</i>	Lactase enzyme, folate, Lactone (buttery and fruity flavour) esters (cyclic) of hydroxyl acids, diacetyl and acetaldehyde.
<i>Leuconostoc</i>	Lactic acid, mannitol (antioxidant). Flavour, lactate (L+), diacetyl and acetaldehyde (Hugenholtz and Smid, 2002).
<i>Monascus purpureus</i>	Lovastatin, identical to monacolin K
<i>Enterococcus faecium</i>	Bacteriocins, enterocin A, enterocin B and enterocin P
<i>Gluconacetobacter xylinus</i>	Acetate, gluconate, acetaldehyde, CO ₂ (Zhang <i>et al.</i> , 2014)
<i>Lactobacillus buchneri</i>	Flavour, lactate, lactic acid, acetic acid and propionic acid (Zhang <i>et al.</i> , 2010)
+ <i>Lactobacillus diolivorans</i>	
<i>Actinobacillus succinogenes</i>	Succinic acid (Zheng <i>et al.</i> 2009). Formate and acetate in high concentrations.
<i>Candida utilis</i>	Lipase and invertase, fatty acids, pyridoxin, (vitamin B ₆), biotin (vitamin B ₇) and single cell protein (Bourdichon <i>et al.</i> , 2012).
<i>Candida milleri</i>	Flavour and texture, Lipases, lactate, acetate, ethanol and CO ₂ (Vigentini <i>et al.</i> , 2014)
<i>Lactobacillus sporogenes</i>	Lactic-acid
<i>Lactobacillus rhamnosus</i>	Exo-polysaccharides (GGISL5 and LCISL3). Pili proteins (SpaCBA) and a sortase.
<i>Lactobacillus casei</i>	β -galactosidase, glycolytic enzymes and able to hydrolyse carbohydrates other than lactose.
<i>Lactobacillus plantarumtarum</i>	Amino acids with new beneficial peptides. Lactate, acetate, acetate kinase, pyruvate oxidase
<i>Bifidobacterium breve</i>	Fructose, lactate and acetate
<i>Propionibacterium sp.</i>	Vitamin B ₁₂ , folate
<i>Geotrichum candidum</i>	Aroma compounds, ethanol, ethyl acetate, ethyl 2-utenoate, ethylbutyrate, ethyl propionate, ethyl esters, 2-hexenoic acid and propanol-1
Mixture of <i>Lactobacillus</i> and yeasts species mainly <i>Saccharomyces</i> and <i>Candida</i> (Microorganisms of sourdough sponges)	Flavour and aroma compounds mainly acetic acid, ethanol, 1-propanol, 2-methyl-1-propanol, ethyl acetate, 3-methyl-1-butanol, 2-methyl-1-butanol, 1-pentanol, 2-methyl-1-pentanol, 1-hexanol, 3-hexen-1-ol, 1-heptanol, 1-octanol acetaldehyde 3-methyl-1-butanol 2-methyl-1-butanol hexanal, 3-methyl-hexanal, heptanal trans-2-heptenal octanal nonanal benzaldehyde diacetyl, hexane, heptane, octane (Salim-ur-Rehman <i>et al.</i> , 2006). Organic acids, niacin, riboflavin, vitamin e, vitamins b ₁ -b ₆ , b ₁₂ , folate, thiamine.

Oda *et al.* (1997) investigated the production of lactic acid from discarded bread (crust) by using an amylolytic bacterium. The researchers demonstrated that the addition of 2% yeast extract in the medium containing 3.58% bread crust caused maximum acid production (Melikoglu and Webb, 2013). The major breakthrough in this research was the conversion of the starch in bakery waste to lactic acid without supplementing starch-degrading enzymes. Leung *et al.* (2012) reported the successful production of succinic acid through the fermentation of waste bread pieces. A novel bio-refinery concept was developed by utilizing waste bread as a sole nutrient source for the production of a nutrient rich feedstock for succinic acid production by *Actinobacillus succinogenes*.

The waste bread was treated by two stage SSF processes: One to produce starch-degrading enzymes and the other to produce proteases. The enzymes were then used to hydrolyse the remaining fraction of the bread waste, as shown in Fig. 3 (Leung *et al.*, 2012). In this process, *Aspergillus awamori* and *Aspergillus oryzae* produce enzyme complexes rich in amylolytic and proteolytic enzymes, respectively. The resulting fermentation outflow was added directly to the bread suspension to generate a hydrolysate containing over 100 g L⁻¹ glucose and 490 mg L⁻¹ free amino nitrogen (Leung *et al.*, 2012).

The bread hydrolysate was used as the sole feedstock for *A. succinogenes* fermentation, which led to the production of 47.3 g L⁻¹ succinic acid. This corresponds to an overall yield of 0.55 g succinic acid per g of bread. This is the highest reported succinic acid yield as compared to the other reported food waste-derived media. The proposed process could be potentially utilized to transform no-value food waste into value added product, succinic acid (Leung *et al.*, 2012).

A summary of the various approaches (enzymatic hydrolysis, ethanol and lactic acid fermentation) adopted by different researchers in the area of waste bread utilization are summarized in Table 1.

Future Trends and Prospects

There is a global desire to divert bread waste from landfill, due to multiple objectives such as limited landfill capacity and increasing cost and numerous side effects such as greenhouse gasses generations. In the recent years, bio processing technologies have made a significant progress to address the challenges associated with bread waste formation. To date, variety of products including ethanol, methane, lactic acid, succinic acid, amylase and protease are generated by fermentation. However, many of these processes are not cost-effective and operationally feasible. Therefore, there is still a need for more sustainable and efficient technologies to enhance the plant profitability from the bread waste bioconversion.

The use of non-pathogenic and non-toxicogenic multifunctional microorganisms with the ability to

produce valuable and high-priced metabolites such as vitamins and bioactive peptides can be seen as a novel approach. Potent microorganisms that can be used for production of these value added products are tabulated under Table 2. These microorganisms are suitable for large-scale industrial fermentation as they possess a high ability to survive the harsh processing conditions (Hugenholtz and Smid, 2002; Desmond *et al.*, 2004; Smith and Jones, 2012; Perricone *et al.*, 2014). Among these microbial sources, lactic acid bacteria seem to be the most important industrial microorganisms. During fermentation, lactic acid bacteria produce a wide range of value added secondary metabolites, some of which have been associated with significant health-promoting properties (NS, 1999; Holzapfel *et al.*, 2001; Sybesma *et al.*, 2003; Leroy and De Vuyst, 2004). In this regard, biosynthesis of antimicrobial substances, aromatic compounds and vitamins by lactic acid bacteria provides an attractive approach for future research trends.

Conclusion

With the advantages of biotechnological processes, microbial strains have been shown to have the ability to convert bread waste into a variety of products. To date, ethanol, methane, lactic acid, succinic acid, amylase and protease have been successfully produced by the SSF and LSF methods. However, there is still a need for innovative and more sustainable bioprocesses to address the issues associated with waste generation and low recycling rates.

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Author's Contributions

All authors equally contributed in this work.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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