

Biotechnological valorization of sugar beet wastes into value-added products

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ABSTRACT

The sugar beet processing in the sugar production industry releases huge amounts of sugar beet pulp, lime residue, and molasses, which can be considered a valuable by-product as a source of cellulose, hemicellulose, and pectin. Sugar beet pulp is often used as a high-energy, low-protein supplement for ruminants to promote optimal rumen health and increase milk production. However, it cannot be used in large quantities and is thrown away, causing environmental pollution. Valorizing sugar beet processing wastes via biotechnological approaches into value-added products is cost-effective and eco-friendly. In this article, recent developments in the biotechnological valorization of sugar beet byproducts to produce biofuels, bioethanol, butanol, biomass and platform chemicals such as gluconic acid, lactic acid, rhamnolipid biosurfactant, and endo-polygalacturonase were reviewed, and the methods provide a way to save the environment. Several sugar beet processing plants in the Kyrgyz Republic can offer these methods as a roadmap for value-added production.

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1. Introduction

Research on sugar's history reveals that sugarcane was initially domesticated in Papua New Guinea. From there, it spread throughout the Pacific and eventually reached India, where crude forms of sugar were produced about 2000 years ago. In the mid-18th century, sugar beet was identified as a viable alternative source of sugar that could be cultivated in temperate regions. [1]. Recently, world sugar production has been approximately 160 Mt yearly, with a per capita consumption of about 23 kg. The chemical composition of commercial sugars produced from cane and beets is sucrose (over 99.5% in white crystalline sugar), even though the crops differ significantly in climatic requirements and photosynthesis pathways [2]. Beet sugar represents only 20%

of the world's sugar production, with the other 80% produced from sugar cane [3]. Sugar beet (*Beta vulgaris*) is an important crop plant in central Europe for sugar production [4]. Due to the climatic conditions of growing sugar beet, it is grown more in Europe than in other countries. The European Union is the world's leading beet sugar producer, with approximately 50% of the total [3]. In Poland, about 11-12 x 10⁹ kilograms of sugar beet is used each year to make white sugar [5]. As a byproduct of this process, around 5.5 x 10⁸ kilograms of sugar beet bagasse (pulp) is obtained, along with lime residue and molasses which are considered waste materials [6]. The technology for producing sugar from beets and generated wastes is shown in Fig. 1.

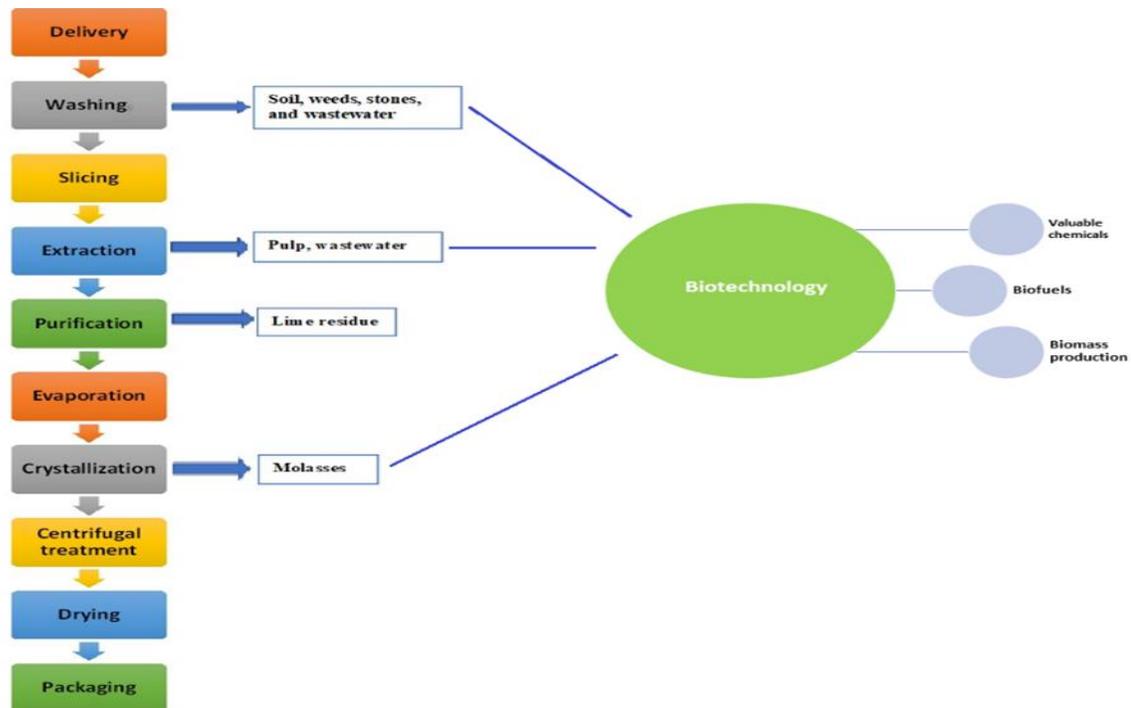


Figure 1. Bioconversion of sugar industrial wastes into value-added products.

Sugar beet pulp (SBP) is a highly fibrous sugar-depleted material produced after sugar is extracted from sugar beet [7]. SBP contains 20-30% cellulose and 18-36% hemicellulose (mainly arabinans), 20-32% pectin, 7-15% protein, and 1-4% lignin (dry weight basis) [8]. Pulp is a valuable cattle feed and supplies carbohydrates, proteins, and minerals. SBP (in dry, pelleted, or ensiled form) is often used as a high-energy, low-protein supplementary food for ruminants promoting optimum rumen conditions and boosting milk production [9].

The lime residue is the residual calcium carbonate precipitate left after the juice purification process, followed by the pressing process. It is a cake-like substance with approximately 70% dry substance (almost the same as soil). It consists of roughly 80% CaCO_3 in its dry substance (DS), 10% inorganic and insoluble organic compounds, and trace elements, such as selenium, zinc, and molybdenum.

Molasses is a thick solution that contains around 50% sucrose and 80% DS along with minerals, vitamins, and organic compounds. Due to its high sugar content and valuable nutrients, it is a popular choice for fermentation processes [7, 10]. It is commonly used in the animal feed industry, as well as in the production of yeast, citric acid, alcohol, rum, and pharmaceuticals [7, 11].

Food wastage has a huge carbon footprint, estimated at 3.3 billion tonnes of CO_2 equivalent to GHG (greenhouse gas) released into the atmosphere annually. The total volume of water used each year to produce lost or wasted food (250 km^3) is equivalent to the annual flow of Russia's Volga River, or three times the volume of Lake Geneva. Similarly, 1.4 billion hectares of land - 28% of the world's agricultural area

- is used annually to produce lost or wasted food [12]. In terms of sugar production, traditional beet sugar processing causes environmental problems mainly due to the elaboration of large amounts of pulp, the consumption of large quantities of lime (which are transformed into sludge), the production of vinasse, and the consumption of energy and water [13]. Due to the huge amount of waste in the production of sugar from beets, it is of great interest to use it as a raw material for producing value-added chemicals such as gluconic acid [14], lactic acid [15], 2,3-butanediol [10], endo- polygalacturonase [16], rhamnolipid [17], propylene glycol [18], biofuels such as butanol [8], hydrogen [19], methane [20], ethanol [21], biomass such as microalgae *Chlorella* [22], single cell protein [23, 6] and sugar beet wastes used in the immobilization of microorganisms [24].

In the Kyrgyz Republic, beets are the only crop for sugar production. It is grown only in the Chui and Talas regions. According to the National Statistical Committee of the Kyrgyz Republic, the area dedicated to sugar beet cultivation decreased in 2022. In the previous year, the republic saw 10.2 thousand hectares of land sown, while in 2022, this decreased to 9.0 thousand hectares, marking an 11.3% decline. Despite this, a total of 468.1 thousand tons of sugar beets were harvested in 2022, with a yield of 518.1 centners per hectare, which is a significant increase of 28% compared to the previous year [25]. According to the Ministry of Economy of the Kyrgyz Republic, the main volume of sugar beet is processed by three enterprises, with an amount of 107.4 thousand tons in 2022. Unfortunately, the waste produced by the sugar industry is not effectively utilized. Currently, sugar producers sell fresh pulp to feed farm animals, but it must be used within 1-2 days due to its quick deterioration. Any

unused pulp is typically buried, leading to soil pollution and environmental damage. After a few weeks, the pulp undergoes oil fermentation, releasing butyric acid into nearby reservoirs.

This work aims to evaluate the biotechnological methods of obtaining value-added products to offer them as a “road map” in the utilization of sugar beet wastes in the Kyrgyz Republic.

2. Valuable chemicals from sugar beet waste

2.1 Gluconic acid

Gluconic acid and its salts are highly sought after for use in various industries such as pharmaceuticals, food, feed, textiles, and leather [26]. There are several methods to obtain gluconic acid, with most being chemical-based. However, there is increasing interest in fermentation as a preferred method for industrial production. In a recent study by Kelleci et al. (2022), three fermentation methods were tested: submerged, semisolid-state, and solid-state fermentations. The results showed that the most efficient method was semisolid-state fermentation, producing 0.354 g of gluconic acid per g of absolute solid substrate. The substrate used was SBP and sugar beet molasses, and it is noteworthy that gluconic acid was produced in situ. In addition, the authors found that CaCO_3 was a suitable neutralizer, and the required calcium carbonate can be obtained from the carbonation cake resulting from sugar juice treatment [14].

2.2 Lactic acid

Lactic acid and its derivatives, including salts and esters, have various applications in the food, polymer, and industrial sectors. They are commonly used in beverages, candies, meat, sauces, and as monomers for producing PLA, which is used to make cups, plates, and utensils. Additionally, lactic acid is utilized in metal plating, cosmetics, and the textile and leather industries [27]. During the fermentation process, lactic acid bacteria produce lactic acid, as demonstrated by a recent study that used *Lactocaseibacillus paracasei* NRRL B-4564 to produce lactic acid using sugar beet molasses [28]. It is worth noting that the MRS medium was modified by adding molasses, and the results were promising. The study found that waste substrate based on sugar beet molasses could substitute for expensive MRS broth as a source of nitrogen, vitamins, minerals, and fermentable sugars for the growth of LAB and LA production. However, another study used granules of SBP to obtain the highest LA concentration (30 g L⁻¹) by *Lactiplantibacillus plantarum*, but pretreatment was required [15].

2.3 Rhamnolipid biosurfactant

Biological surface-active agents, also known as biosurfactants, are produced by various microorganisms and have unique properties such as lowering surface tension, increasing solubility of poorly soluble compounds, non-toxicity, non-allergenicity, and biodegradability [29]. One example of biosurfactant is the rhamnolipid, which is

commonly produced by *Pseudomonas aeruginosa*. In a study by Onbasli and Aslim (2009), the same microorganism was used but with sugar beet molasses as a substrate [17]. It was found that *Pseudomonas* spp. can use molasses as a carbon source for rhamnolipid production without any pretreatment. The molasses was simply diluted with distilled water to the required concentration, the pH was adjusted to 7.0, and then sterilized in an autoclave.

2.4 Endo-polygalacturonase

It is crucial to note that endo-polygalacturonase (endo-PG), an enzyme derived from agroindustrial waste SBP, holds immense potential in various industrial applications. This enzyme serves as an efficient raw carbon source for breaking down industrial waste during urban waste disposal. A recent study by authors [16] conducted submerged fermentation using *A. niger* and *P. variotii* as fermentation agents, with SBP being dried and milled as the solitary pretreatment. The study demonstrated that SBP is a promising inducer for endo-PG production. However, it is imperative to test all types of fermentation conditions to determine the most effective method for producing commercial enzymes while optimizing the enzyme production and extraction methods from different industrial wastes. The results of this study have significant implications in the food, pharmaceutical, and other industrial sectors [16].

2.5 2,3-Butanediol

The chemical 2,3-Butanediol shows promise as a versatile substance with various potential uses such as in the production of printing inks, perfumes, synthetic rubber, fumigants, antifreeze agents, fuel additives, food products, and pharmaceuticals [30]. Recently, researchers [10] obtained 2,3-Butanediol through fermentation using *Bacillus amyloliquefaciens* TUL 308. The study found that sugar beet molasses was the most suitable carbon source for the synthesis of 2,3-Butanediol by the tested strain. This carbon source is not only cost-effective but also requires no pretreatment before use.

3. Biofuels

3.1 Butanol

The relevance of biofuel has increased significantly in recent times due to the depletion of fossil fuel sources and their negative impact on the environment. In the current century, there is a growing interest in waste management and research on converting waste to green energy [31]. As a result, there is a need for crude oil that is environmentally friendly and provides energy similar to conventional diesel. Butanol is a liquid fuel that is considered a green energy and offers several advantages over ethanol, including an energy value similar to gasoline, lower corrosiveness, low vapor pressure, lower miscibility with water, high flash point, and easy transportation through existing pipelines. Additionally, it can

reduce hydrocarbon emissions by 95% and nitrogen oxides by 37% [8]. Butanol has the potential to address some of the infrastructure problems related to fuel cell use and can be dispersed through existing pipelines and filling stations. It offers a safer fuel with more hydrogen, making it a promising alternative energy source [32]. Table 1 displays the results of a study conducted by the authors [8], who used acetone-butanol-ethanol (ABE) fermentation to produce butanol. This process produces acetone, butanol, and ethanol in a 3:6:1 ratio, with butanol being the main product. The feedstock used for ABE fermentation was SBP and *Clostridium beijerinckii*. The scientists highlighted the significance of pretreatment and compared various methods such as dilute sulfuric acid pretreatment, autohydrolysis pretreatment at pH 4, and enzymatic hydrolysis. Autohydrolysis at pH 4 (120°C, 6% SBP (w/w), 5 minutes) was found to be the most effective pretreatment for enhancing overall sugar release yields in enzymatic hydrolysis and acetone and butanol yields in the fermentation process. It has advantages over conventional dilute acid pretreatment, such as higher solid recoveries, no washing needs, and lower use of chemicals. The authors claim that overall yields of 143.2 g ABE/kg SBP (62.3 g acetone and 80.9 g butanol) can be achieved.

3.2 Hydrogen

Hydrogen is a valuable commodity in various industries due to its versatility. It is not only highly efficient but also an eco-friendly fuel. When hydrogen is burned, it does not emit greenhouse gases, ozone-depleting chemicals, acid rain components, or any pollutants [33]. Moreover, it is often suggested as a safe fuel since it only produces water upon combustion [34]. In a study by authors [19], single-stage photofermentation was applied under anaerobic conditions using sugar beet molasses and *Rhodobacter capsulatus* JP91 as inoculum. Without any pretreatment, beet molasses yielded 10.5 mol H₂/mol sucrose, as shown in Table 1. Although two-stage systems were previously deemed the most efficient for hydrogen production, this paper carried out single-stage photofermentation, which saves space and energy. The single-stage system also produces more hydrogen at a low sugar concentration of 1 g sugar/L, which makes up for any deficiency in hydrogen production.

3.3 Methane

Organic waste, such as sugar beet wastes, can be effectively utilized for biogas production through anaerobic digestion. This process is currently the primary method for obtaining gas from organic waste. Anaerobic digestion occurs naturally in various environments, such as marshes, bogs, landfills, and dedicated digesters. It involves the conversion of organic waste into a combustible biogas, which can be used as a boiler or motor fuel, or even upgraded to pipeline quality [35]. Researchers have analyzed various scientific papers on sugar production waste and discovered positive outcomes. One study [36] examined the co-digestion of waste with SBP and wastewater using anaerobic seed culture from a municipal wastewater treatment plant as inoculum. The

addition of wastewater led to an increase in methane production rate for the beet pulp, rather than an increase in ultimate biodegradability. Additionally, the wastewater replaced fresh water typically used as a diluent for pulp digestion. It's important to note that pretreatment is crucial for anaerobic digestion, which was also addressed in this study. This involved physically treating or cleaning the wastewater and drying and crushing the beet pulp. It's worth noting that not only is beet pulp utilized, which is typically used for animal feed, but also wastewater from sugar production that is often discarded. This helps save on the fresh water needed for fermentation. In a similar study, other experts [20] incorporated sugar beet waste as an extra carbon source in anaerobic co-digestors with cow and pig manure. It was found that the co-digestion with manure significantly reduced the inhibitory effect of volatile fatty acids at high organic loading rates, leading to a 70% and 31% increase in methane production for pig and cow manure co-digestion, respectively, compared to individual digestion of sugar beet byproducts.

3.4 Bioethanol

Bioethanol is a renewable biofuel made from various biomass materials that can be used as a sole fuel source or partial substitute for fossil fuels. Bioethanol production from agro-industrial byproducts, residues, and wastes is one example of sustainable energy production [21, 37]. Several studies have explored the production of bioethanol and identified effective processing methods. Dilute acid pretreatment, for instance, has been found to significantly improve the enzymatic hydrolysis and ethanol yield of SBP. Under the optimum conditions, the ethanol yield from pretreated SBP in a simultaneous saccharification and fermentation process using *Escherichia coli* KO11 was 0.4 g ethanol/g dry matter [21]. Additionally, ensiling SBP has been shown to increase its reducing sugar yield upon enzymatic hydrolysis, but it requires water washing. Authors report that ensiled SBP does not require sterilization for fermentation with *E. coli* KO11, and washing it decreases ethanol yield. This suggests that ensilage could be a useful method for storing and pretreating biomass to enhance biofuel yield [38]. In another study, researchers found that the liquid fraction obtained from the hydrolysis of SBP could be subjected to alcoholic fermentation, while the remaining solid residue and stillage were used for methane or hydrogen production [39]. Bioethanol fermentation using the coculture of *Saccharomyces cerevisiae* Ethanol Red and *Scheffersomyces stipitis* LOCK0047 resulted in 12.6 g/L ethanol, as shown in Table 1. The largest hydrogen yield (252 dm³ H₂/kg VS) was achieved with sugar beet stillage (SBS) that underwent thermal pretreatment and had its inoculum pH adjusted, and the maximum methane yield was 444 dm³ CH₄/kg volatile solids (VS). As a result, the stillage obtained after alcoholic fermentation was not discarded but used as a raw material for the production of methane or hydrogen, making this process economically viable and environmentally friendly.

4. Biomass production

4.1 Microalgae *Chlorella*

The cultivation of *Chlorella* for producing biomass and derivative products is an industrial activity that has already been established commercially in several countries. The interest in these microalgae is due to their rapid growth and simple life cycles, allowing in-depth studies of their mechanisms and use as a food substitute in terms of their high protein, carotenoid, vitamin, and mineral contents [40]. *Chlorella* has a high protein content (58 g/100g) compared with wheat (13 g/100 g), chicken (24 g/100 g) and fish (18-24 g/100 g) [41]. Wang et al. (2019) conducted a study where they utilized SBP as an illustration of lignocellulosic biomass. This biomass was nonairtightly fermented with digested dairy manure to offer additional organic carbon sources for microalgae cultivation [22]. Additionally, the authors reported that *Chlorella* cultured in the 3-fold diluted hydrolysate demonstrated the best growth and nutrient reduction performance, in which case the final biomass density reached 2.17 g/L.

4.2 Single-cell protein

After distilling beet molasses to make alcohol, a dark brown liquid called vinasse is produced. This liquid is high in organic matter and salt content, making it difficult to dispose of and environmentally concerning [42, 23]. Unlike other sugar production wastes, beet vinasse is not often suitable for further processing. However, researchers have found that beet vinasse obtained after fermentation can be used to produce valuable single-cell protein and *Spirulina platensis* biomass. Single-cell protein is a type of protein extracted from cultivated microbial biomass that can be used to supplement diets by replacing costly conventional protein sources like soymeal and fishmeal [43]. In a study conducted by Coca et al. (2015), beet vinasse was used to supplement a mineral medium in a vertical airlift photobioreactor [23]. The researchers noted that betaine, a nitrogenous compound found in beet vinasse, was completely removed from the broth due to its utilization as a nutrient source by the microalgae. Adding 1 g L⁻¹ vinasse to the mineral medium significantly increased biomass and protein productivity compared to an unsupplemented medium. In another study, researchers [6] utilized SBP as a substrate for fed-batch cultivation of *C. tropicalis* to convert it into SCP. However, they highlighted the significance of pretreatment to ensure the culture is abundant in valuable components. The resulting biomass had a protein content of 52.3% of DM, indicating that SBP can be effectively utilized to produce yeast biomass that contains noteworthy amounts of SCP.

4.3 Immobilization agent

Cell immobilization refers to the process of preventing cells from moving either naturally or artificially [44]. This technique can be utilized to produce various value-added products, including biopharmaceuticals, bioplastics, biofuels, and bioremediation. Additionally, it can be employed in the development of biological biosensors for tissue regeneration in the medical field [45]. In a study on bioethanol production, *Saccharomyces cerevisiae* was immobilized using SBP [24]. The researchers found that this method enabled the rational use of intermediates and byproducts of sugar beet processing, contributing to the zero-waste goal. They achieved a maximum ethanol yield of 0.446 ± 0.017 g/g, with beet thick juice being the best substrate for ethanol production, requiring no nutrient supplementation. Autoclaving proved the supports to be both mechanically and chemically stable. The researchers confirmed that SBP was an ideal material for immobilizing *S. cerevisiae* due to its porosity, high water swelling capacity, biocompatibility, and high cell retention capacity. The immobilization method was deemed inexpensive, simple, and easy, with the exploited dried SBP supported with immobilized yeast cells potentially serving as a protein-enriched complement for animal feed. In a recent study [46], researchers explored a method for immobilizing *Leuconostoc mesenteroides* T3 using SBP, while utilizing beet molasses as a nutrient source for dextransucrase production. Dextransucrase (DS) enzymes, primarily secreted by *Leuconostoc*, *Streptococcus*, and *Lactobacillus* species, synthesize the majority of dextrans in nature [47]. The authors of the study [46] found that immobilizing the cells onto SBP enhances DS production. The results of the repeated batch fermentation experiment with *Lc. mesenteroides* T3 immobilized onto the SBP-NaOH carrier showed that four subsequent cycles could be performed with immobilized cells, until the productivity decreased by approximately 60%.

5 Conclusions

The field of biotechnology has gained prominence for its ability to extract valuable products from waste materials. In Europe, where there is a high volume of sugar production, sugar factories have found ways to recycle their waste as substrates, resulting in the creation of valuable products and energy. Various studies have integrated different types of agro-waste into a biorefining process, with sugar beet waste playing a crucial role as a carbon source. It's important to note that pretreatment is essential, but it's not always necessary, depending on the desired outcome. These findings demonstrate that seemingly useless sugar waste can be transformed into valuable products like biofuels, chemicals, immobilization agents, and biomass. These solutions can be implemented in sugar factories across the Kyrgyz Republic.

Table 1. List of some value-added products using sugar beet byproducts as a fermentation substrate.

Sugar beet wastes	Pretreatment	Pretreatment process	Method	Microorganism/Inoculum	Obtained value-added product	Ref.
Sugar beet molasses	No		Fermentation/three batch cultures	<i>Bacillus amyloliquefaciens</i> TUL 308	2,3-butanediol	[10]
SBP	Yes	Drying and milling to a powder	Submerged fermentation (static culture)	<i>Aspergillus niger</i> , <i>Paecilomyces variotii</i>	Endo-polygalacturonase	[16]
Sugar beet molasses/distillery stillage	No	Sugar beet molasses were used as modified MRS media	Batch cultures, with shaking and under anaerobic conditions	<i>Lactocaseibacillus paracasei</i> NRRL B-4564	Lactic acid	[28]
SBP/sugar beet molasses	Yes	Drying	Semisolid state fermentation	<i>Aspergillus niger</i> NRRL-3	Gluconic acid	[14]
Sugar beet molasses	No		Fermentation in flasks	<i>Pseudomonas spp.</i>	Rhamnolipid biosurfactant	[17]
Sugar-beet pulp	Yes	Autohydrolysis at pH 4 (120 °C, 6% SBP (w/w), 5 minutes)	ABE fermentation (anaerobic fermentation)	<i>Clostridium beijerinckii</i>	Acetone, butanol	[8]
Sugar beet molasses	No		Single stage photofermentation (anaerobic conditions)	<i>Rhodobacter capsulatus</i> JP91	Hydrogen	[19]
SBP, sugar beet molasses	No		Anaerobic codigestion	Inoc1-municipal solid waste, Inoc2- the effluent from the reactor of single digestion	Methane	[20]
SBP, wastewater	Yes	Physical pretreatment/SBP was dried and homogenized to powder	Anaerobic mesophilic codigestion	Anaerobic seed culture from the municipal wastewater treatment plant	Methane	[36]
SBP	Yes	Dilute acid pretreatment and Enzymatic hydrolysis	SSF (solid-state fermentation)	<i>E. coli</i> KO11	Fuel ethanol	[21]
SBP	Yes	Enzymatically depolymerization	Bioethanol fermentation, anaerobic digestion, dark fermentation	<i>Saccharomyces cerevisiae</i> Ethanol Red, and <i>Scheffersomyces stipitis</i> /Sewage sludge	Bioethanol, methane, hydrogen	[39]
SBP	Yes	Anaerobic digestion dairy manure	Nonairtight fermentation	<i>Chlorella</i> seeds	<i>Chlorella</i>	[22]
Sugar beet vinasse	No		Fermentation in a vertical airlift tubular photobioreactor	<i>Spirulina platensis</i>	Single cell protein	[23]
SBP	Yes	Enzymatic hydrolysis	Fed-batch cultivation	<i>Candida tropicalis</i> LOCK 0007	Single cell protein	[6]
SBP	Yes	Drying	Repeated batch ethanol fermentation	<i>Saccharomyces cerevisiae</i>	Bioethanol	[24]
SBP/sugar beet molasses	Yes	Alkaline pretreatment with NaOH of SBP	Fermentation in Erlenmeyer flasks	<i>Leuconostoc mesenteroides</i> T3	Dextranucrase (DS)	[46]

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