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Review

Biofuels from inulin-rich feedstocks: A comprehensive review

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HIGHLIGHTS

- Inulin-rich feedstocks can be explored for bioethanol, acetone and butanol production.
- Jerusalem artichoke is a potent inulin-rich feedstock for biofuels production.
- Consolidated bioprocessing is an efficient strategy for biofuels production.
- Bioprocessing strategies limitations are the key challenges for biofuels commercialization.

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ABSTRACT

Biofuels are considered as a pre-eminent alternate to fossil fuels to meet the demand of future energy supply in a sustainable manner. Conventionally, they are produced from lignocellulosic raw materials. Saccharification of lignocellulosic raw materials for bioethanol production is a cumbersome process as compared to inulin-rich feedstocks. Various inulin-rich feedstocks, viz. jerusalem artichoke, chicory, dahlia, asparagus sp., etc. has also been exploited for the production of biofuels, viz. bioethanol, acetone, butanol, etc. The ubiquitous availability of inulin-rich feedstocks and presence of large amount of inulin makes them a robust substrate for biofuels production. Different strategies, viz. separate hydrolysis and fermentation, simultaneous saccharification and fermentation and consolidated bioprocessing have been explored for the conversion of inulin-rich feedstocks into biofuels. These bioprocess strategies are simple and efficient. The present review elaborates the prospective of inulin-rich feedstocks for biofuels production. Bioprocess strategies exploited for the conversion of inulin-rich feedstocks have also been highlighted.

1. Introduction

The demand of global energy requirements from fossil fuels is continuously increasing, due to the tremendous increase in the global population. Fossil fuels are the natural fuel sources which were formed from the remains of plants and animals that were buried million years ago. Now-a-days, these are not considered as a tenable energy source from environmental and economical point of views. The major drawbacks of using fossil fuels are air and water pollution, non-renewable source of energy and global warming world-wide. Therefore, the quest for environmentally safe and sustainable source of energy to fulfil the demands of consumer societies and industrial economy has become imperative in the recent years. Biofuels are an alternate substitute of fossil fuels and their major advantages of use are the reduced

environmental pollution and renewable energy source. Renewable energy sources could play a key role in replacing the petroleum-based fuels that are used for generating heat and electricity as well as for transportation purposes.

Biofuels are produced from numerous renewable sources that can be either a plant material or an animal waste. Inulin-rich feedstocks are an important renewable energy source which can also be exploited for biofuels production like bioethanol, acetone, butanol, etc. Different inulin enriched feedstocks like chicory, asparagus sp., jerusalem artichoke, dahlia, *Agave tequilana*, etc. are widely explored for biofuels production (Singh et al., 2019; Singh et al., 2020), fructose syrup (Singh et al., 2017; 2018a,b), fructooligosaccharides (Singh et al., 2016; Singh and Singh, 2010, 2019a,b), etc. Amongst these, jerusalem artichoke is a potential vegetable source and it has been widely used for biofuels

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production. Jerusalem artichoke is considered as the optimistic crop of future in Europe, China and New Zealand. Inulin accounts >80% of total carbohydrates present on fresh weight basis of Jerusalem artichoke tubers. The ubiquitous availability of Jerusalem artichoke in both tropical and temperate countries across the world, high resistance towards pests and drought conditions and increased solubility at high temperature with a clear solution, makes it a potent substrate for biofuels production. Besides, both asparagus and dahlia tubers are also an important source of inulin which can also be explored for biofuels production. Inulin-rich feedstocks are potent substrates for the production of biofuels. Amongst them Jerusalem artichoke is widely cultivated worldwide and largely explored for the production of biofuels at laboratory scale. However, efforts should be made to increase the cultivation area of other inulin-rich feedstocks like dahlia, chicory, asparagus, etc. The problems associated with strain improvement, substrates availability and bioprocesses feasibility are the major challenges for biofuels production from inulin-rich feedstocks. It is expected in near future that a sustainable and cost-effective bioprocess for biofuels production would be developed by understanding the scientific fundamentals and engineered principles. Strict planting regulations and government policies may increase the usage of inulin-rich feedstocks for biofuels production. Researchers have to understand the barriers of inulin-rich feedstocks for biofuels production. The present review describes the prospective of inulin-rich feedstocks for biofuels production, viz. bioethanol, butanol, acetone, etc. Various bioprocess strategies, viz. separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF) and consolidated bioprocessing (CBP) used for the conversion of inulin-rich feedstocks into biofuels has also been elaborated.

2. Inulin-rich feedstocks – A robust substrate for biofuels production

Inulin is a robust dietary fibrous fructan type polysaccharide which was first discovered from the roots of *Inula helenium* belonging to family *Asteraceae* (Rose, 1804). It is symbolized as GF_n, where 'G', 'F' and 'n' represents glucose, fructose and number of fructose units, respectively. Inulin molecules has a number of fructose units which are linked to each other by β-D (2 → 1) glycosidic linkages and glucose moiety is linked to

fructose unit by α-D (1 → 2) glycosidic linkage at its reducing end. However in a few cases, fructose moiety has also been reported at the reducing end of inulin (Hébet et al., 1998). It is a natural storage carbohydrate present in the bulbs and tubers of numerous families of plants viz., *Campanulaceae*, *Boraginaceae*, *Primulaceae*, *Liliaceae*, *Malpighiaceae*, *Asteraceae*, *Violaceae*, *Poaceae*, *Amaryllidaceae*, *Styracaceae*, etc. (Table 1). Likewise few daily use fruits and vegetables like *Solanum lycopersicum* (tomato), *Musa* (banana), *Triticum aestivum* (wheat), *Avena Sativa* (oat), etc. also contain inulin, but in a very less quantity. Inulin is primarily produced from *Cichorium intybus* (chicory) root tubers, *Helianthus tuberosus* (Jerusalem artichoke) tubers and *Dahlia pinnata* (dahlia) tubers. Amongst these, Jerusalem artichoke also called as sunchoke/sunroot is a potential inulin-rich feedstock which contains approximately 16–23% inulin (Bach et al., 2015). It is considered as a vigorous substrate for biofuels production like bioethanol, acetone, butanol, etc. Jerusalem artichoke is native to North America which later spread to European countries in the 17th century (Ma et al., 2011). It is now cultivated world-wide especially in China and Israel, due to its large economic value in various fields. The economic value of Jerusalem artichoke mainly depends upon its tubers which are rich in carbohydrates. Out of the total carbohydrate present in tuberous roots of Jerusalem artichoke, inulin accounts >80% along with small quantity of free fructose, kestose, sucrose and glucose (Gao & Yuan, 2019). In Jerusalem artichoke tubers apart from inulin, a number of soluble proteins, amino-acids and minerals are also transformed into bioethanol during bioprocessing (Zhou et al., 2008). Due to the resistance of Jerusalem artichoke towards different microbes and pests, drought and frost tolerance, it can be easily cultivated in different types of soils with a little bit of fertilizers. Being more tolerant to harsh conditions Jerusalem artichoke can be potentially cultivated in cold countries like Scandinavia, Finland, and Iceland (Slimestad et al., 2010). The economics of production cost of Jerusalem artichoke tubers for bioethanol production in USA, Canada and Australia has been reported recently (Bedzo et al., 2020). Jerusalem artichoke tubers production cost/hectare for bioethanol production has been reported US\$ 5300 in USA, US\$ 2900 in Canada and US\$ 1080 in Australia, by these researchers. Probably, the abundant availability of Jerusalem artichoke tubers makes them the cheapest bioethanol producing feedstock for cost-effective bioethanol production (20 €/ton) as

Table 1

Inulin-rich feedstock	Scientific name	Inulin containing part	Inulin (%) ^a	Reference/s
Onion	<i>Allium cepa</i>	Bulbs	2–6	De Leenheer, 1996; Van Loo et al., 1995
Burdock	<i>Arctium</i> sp.	Roots	3.5–4.0	De Leenheer, 1996; Van Loo et al., 1995
Leek	<i>Allium ampeloprasum</i> var. <i>porrum</i>	Bulbs	3–10	De Leenheer, 1996; Van Loo et al., 1995
Garlic	<i>Allium sativum</i>	Bulbs	9–16	De Leenheer, 1996; Van Loo et al., 1995
Safed musli	<i>Asparagus racemosus</i>	Root tubers	10–15	Gupta and Kaur 1997
Agave	<i>Agave americana</i>	Lobes	7–10	Partida et al. 1998
Shatwaar	<i>Asparagus officinalis</i>	Root tubers	10–15	Gupta and Kaur 1997
Camass	<i>Camassia</i> sp.	Bulbs	12–22	De Leenheer, 1996; Van Loo et al., 1995
Artichoke	<i>Cynara cardunculus</i>	Leaves-heart	3–10	De Leenheer, 1996; Van Loo et al., 1995
Chicory	<i>Cichorium intybus</i>	Roots	15–20	De Leenheer, 1996; Gupta and Kaur, 1997; Van Loo et al., 1995
Yam	<i>Dioscorea esculenta</i>	Root tubers	7.5	Handayani et al. 2016
Dahlia	<i>Dahlia</i> sp.	Root tubers	15–20	Gupta and Kaur 1997
Globe thistle	<i>Echinops ritro</i>	Roots	NS	Vergauwen et al. 2003
Jerusalem artichoke	<i>Helianthus tuberosus</i>	Roots	16–23	Bach et al. 2015
Sunflower	<i>Helianthus annuus</i>	Root tubers	NS	Chi et al. 2011
Barley	<i>Hordeum vulgare</i>	Grains	NS	Van Loo et al. 1995
Lettuce	<i>Lactuca sativa</i>	Roots	NS	Hendry and Wallace 1993
Banana	<i>Musa acuminata</i>	Fruits	0.3–0.7	Van Loo et al. 1995
Murnong	<i>Microseris lanceolata</i>	Roots	8–13	Van Loo et al. 1995
Suma	<i>Pfalia glomerata</i>	Roots	11.45 ^b	Caleffi et al. 2015
Spanish salsify	<i>Scorzonera hispanica</i>	Roots	8.15–10.75	Dolota and Dąbrowska 2004
Kuth	<i>Saussurea lappa</i>	Roots	18–20	Kuniyal et al. 2005
Yacon	<i>Smallanthus sonchifolius</i>	Roots	3–19	Van Loo et al. 1995
Rye	<i>Secale cereal</i>	Grains	0.5–1.0	Van Loo et al. 1995
Sweet leaf	<i>Stevia rebaudiana</i>	Roots	18–23 ^b	Lopes et al. 2015
Salsify	<i>Tragopogon</i> sp.	Roots	15–20	Gupta and Kaur 1997
Dandelion	<i>Taraxacum officinale</i>	Leaves	12–15	Van Loo et al. 1995

NS: Not specified; ^aFresh biomass (%); ^bDry biomass (%)

compared to other feedstocks like cassava (820 €/ton) (Song et al., 2017). Ethanol yield (2500–6500 L/ha) from Jerusalem artichoke tubers in comparison to corn (4182 L/ha) and sugarcane (6471 L/ha) has been reported (Goldemberg and Guardabassi, 2010). Numerous studies have shown that hydrolysis of Jerusalem artichoke tubers can yield (3060–11,000 L/ha) of ethanol (Goldemberg and Guardabassi, 2010) which is comparatively higher than sugarcane (6471 L/ha in Brazil) and corn (4182 L/ha in USA) (Goldemberg and Guardabassi, 2010). Chicory is another important edible inulin-rich feedstock which contains approximately 15–20% inulin in its fleshy roots along with other soluble and insoluble carbohydrates (Vandamme and Derycke, 1983). It is a widely cultivated plant used for the production of inulin (El-Kholy et al., 2020) and biofuels (Wang et al., 2016; Ujor et al., 2015). Chicory roots are considered as rich fibre source with various dietary and medicinal uses (Vandoorne et al., 2012). Upon maturation of chicory root tubers, the catalytic action of inulinases on inulin produces oligofructose. Hence, chicory is an abundant source of oligofructose as well as inulin. As per the report of Markets and Markets (<https://www.marketsandmarkets.com/Market-Reports/chicory-market-122013320.html>), chicory market size globally was estimated around 685 million US\$ in 2020 and it is expected to increase around 905 million US\$ by 2025. In India, chicory is mainly cultivated in two states, viz. Gujarat and Uttar Pradesh and it accounts alone approximately 97% of the total chicory produced in India. Asia Pacific regional market exhibits lucrative opportunities of cultivation of chicory in future (<http://niftem.ac.in/newsite/pmfm/wp-content/uploads/2021/10/chicorywriteup.pdf>). Dahlia a perpetual flourishing ornamental plant is another significant inulin-rich feedstock. It is also a potent candidate for biofuels production. It is distinguished on the basis of colour and inflorescence pattern and it is one of the most popular garden plant cultivated world-wide (McClaren, 2009). Dahlia root tubers are rich source of inulin (15–20%) and are considered as potent inulin-rich feedstock for production of biofuels. The growth and the quality of tubers depends upon the type of soil and nutrients availability (Tariq et al., 2012). At present, there are 41 international associations concerned with the cultivation and spreading of dahlia. Asparagus is another perennial herb which contains approximately 10–15% inulin in its tuberous roots. In addition to inulin, *Asparagus racemosus* and *Asparagus officinalis* root tubers also contains minerals (Mg^{2+} , Zn^{2+} , Se^{2-}), vitamins (E and C), glutathione and fructooligosaccharides. Due to the presence of all these nutrients, asparagus is considered as a potent inulin-rich feedstock for biofuels production. In United States, asparagus is cultivated in 20,000–25,000 acres with a value of 70–100 million US\$ annually. The largest asparagus-producing states are Michigan, California, New Jersey and Washington. Most of the asparagus harvested in the United States is sold as fresh produce (<http://extension.psu.edu/asparagus-production>).

The yield of inulin from inulin-rich feedstocks largely depends upon their cultivation strategies used, environmental conditions and harvesting period of a crop. The annual harvesting of inulin-rich feedstocks is the only possible way to achieve a high inulin yield world-wide which can be further converted into biofuels using different strategies. Inulin's degree of polymerization (DP) have an important role in its operational functionality. Inulin obtained from plant sources usually have low DP (20–60) with low molecular weight (Singh & Singh, 2010). Whereas, inulin obtained from bacterial and fungal sources like *Bacillus*, *Aspergillus*, *Streptococcus*, etc. have high DP (<10,000) with high molecular weight (Franck and Leenheer, 2006). The DP of plant inulin varies according to their species, maturation at harvest time, climate conditions, age of plant, storage time and temperature after harvesting (Singh and Singh, 2017). Long term storage causes degradation of high molecular weight carbohydrate moieties which lowers down the DP of plant inulin. The solubility of inulin is also dependent upon its DP. Low DP oligomers are easily soluble in water than high DP oligomers. The increase in temperature increases the hydrophilic nature of inulin. This is due to the increase in the intermolecular voids of water with elevated temperature.

3. Strategies for biofuels production from inulin-rich feedstocks

Inulin enriched feedstocks are a renewable source to produce different microbial products, viz. biofuels, inulinases, fructooligosaccharides, high fructose syrup, etc. (Singh et al., 2019). Inulin can be used directly in its inherent form for the preparation of microbial products, but due to its high cost, inulin-rich feedstocks are an effectual alternative to pure inulin. These can be exploited directly for the preparation of biofuels using different bioprocess strategies, viz. SHF, SSF and CBP.

3.1. Separate hydrolysis and fermentation (SHF)

Hydrolysis process involves conversion of a carbohydrate polymer into fermentable sugars. This step is essential before fermentation to release the fermentable sugars. SHF is carried out into two separate vessels. It involves hydrolysis of inulin of inulin-rich feedstocks to release the fermentable sugars which is further followed by fermentation of the released sugars to produce biofuels using a appropriate microbial strain. The independent operation of both hydrolysis and fermentation at their optimal conditions is the foremost advantage of this bioprocess strategy. The other advantage of SHF is the possibility to operate the fermentation process in a continuous mode by cell recycling. However, the major drawbacks of SHF are feedback inhibition by the released end product of a reaction and chances of contamination, due to extended residual time of hydrolysis process. The hydrolysis of inulin-rich feedstocks can be performed by two methods namely: (i) acidic hydrolysis and (ii) enzymatic hydrolysis:

3.1.1. Acidic hydrolysis

In acidic hydrolysis, inulin-rich feedstocks are hydrolysed in the presence of dilute or concentrated acids like HCl, H_2SO_4 , etc. Due to the corrosion and environmental hazards, dilute acid hydrolysis is preferred over the hydrolysis by concentrated acid. The hydrolysis with dilute acid is performed at high pressure and temperature within a short span of time which results in the hydrolysis of inulin of a inulin-rich feedstock. However, harsh conditions like concentrated acid together with high pressure and temperature will require an additional downstream neutralization cost which makes the process costly (Su et al., 2006). The cost of dilute acid hydrolysis of inulin is low, but the process is rather complicated as the hydrolysates often contain 5-hydroxymethylfurfural, a microbial growth inhibitor (Lujan-Rhenals et al., 2014). Therefore in a bioprocess, enzymatic hydrolysis is preferred over acidic hydrolysis of inulin.

3.1.2. Enzymatic hydrolysis

Inulin-rich feedstocks are hydrolysed by inulinases from numerous microbial strains during enzymatic hydrolysis. Enzymatic hydrolysis is preferred under mild conditions (pH 4.5–6.5 and 40–50 °C). It has two major advantages, i.e. low utility cost due to low corrosion problems and environmental friendly process, due to less toxicity of the hydrolysates. The major drawback of this process is the high cost of enzyme, long residual time and feedback inhibition (Taherzadeh and Karimi, 2007). Though, many researchers still consider enzymatic hydrolysis a cost-effective bioprocess to produce ethanol in the long run, as compared to acidic hydrolysis (Hamelinck, et al., 2005; Horn and Eijssink, 2010). The efficiency of enzymatic hydrolysis is influenced by many factors like surface properties of substrate (degree of polymerization and crystallinity), hydrolysis condition (pH, temperature and enzyme concentration) and also the factors involved in the enzyme mechanism and interaction (Taherzadeh and Karimi, 2007).

3.2. Simultaneous saccharification and fermentation (SSF)

SSF is a bioprocess which involves the simultaneous hydrolysis and fermentation of inulin enriched feedstocks in a single vessel using either

a single or co-culture of micro-organisms. The major advantages of SSF are reduced feedback inhibition and less costly process. Immediate utilization of released fermentable sugars, less time consumption and increased productivity are the other advantages of this process. SSF is preferred over SHF, due to increased inhibitors tolerance, utilization of high substrate and biocatalyst concentration in the same reactor and it also reduces chances of contamination. In SSF, immediate consumption of the released sugars does not lead to their accumulation, which results in continuous operation of the biocatalyst which increases the product yield. Moreover, performing both hydrolysis and fermentation in a single vessel reduces the process time and unit operations cost (Hans et al., 2019). However, the major drawback of SSF is to find suitable optimal operational conditions for both hydrolysis and fermentation process. The another drawback of SSF is the inability to recycle both biocatalyst and fermenting microorganism for their repeated use. Different process variables like temperature, pH, fermentation time, substrate concentration and biocatalyst concentration influence the product yield during SSF. The medium components must also be screened for potential carbon, nitrogen, phosphorous and potassium source to enhance the productivity during SSF (Kanagasabai et al., 2019).

3.3. Consolidated bioprocessing (CBP)

In consolidated bioprocessing, all aspects like enzyme production, enzymatic saccharification of the substrate and fermentation proceeds simultaneously in a single vessel using either a single or consortium of micro-organisms. This results in enhanced efficiency of bioprocess, eliminates the additional requirement of a hydrolytic enzyme and lowers down the feedback inhibition of the biocatalyst by the released sugars (Olson et al., 2012). CBP is a promising technique which utilizes few reactor vessels and offer simplicity in operating procedure by minimizing the requirement of biocatalyst and fermentation step, and thus circumvents both investment and maintenance cost (Mbaneme-Smith and Chinn, 2015). In conventional systems, released sugars inhibits the saccharification process, while in CBP fermentation step converts these sugars into a desired product before they become inhibitory during the hydrolysis step (Salehi Jouzani and Taherzadeh, 2015). Microorganisms with diverse range of characteristics, i.e. optimal substrate concentration utilization and product forming properties like increased hydrolysis rate and enhanced productivity are preferred in CBP. The selection of a suitable thermophilic microorganism can increase the reaction rate is the major challenge, while conducting CBP at ambient conditions for a mesophilic microorganism. Although the rational as well as potential of CBP for biofuels production do exist, the sensitivity of microorganism towards alcoholic solvents, limited microbial growth from the saccharified sugar hydrolysate and prolonged fermentation time, have focused the research on increasing the robustness of microorganisms for their use in CBP. The use of genetically modified microorganisms can overcome these limitations. Although, biochemical conversion process justify its potential for the production of biofuels with minimal environmental impact. The present day challenges are to efficiently convert inulin of inulin-rich feedstocks into sugars and processing these fermentable sugars into a desired biofuel using a potential microbial strain with high productivity and low operation and maintenance cost. All of these can be fulfilled by using an efficient CBP strategy.

Amongst the various bioprocessing strategies used for the pre-treatment of inulin-rich feedstocks, CBP is considered as a promising strategy for efficient biofuel production, due to the combination of three processes simultaneously, viz. enzyme production, saccharification and fermentation. This strategy can reduce the reactor and the enzyme cost, which are the major impediments to low-cost biomass processing. Despite of the potential advantages of CBP, its few limitations have not allowed the usage of this strategy for industrial bioethanol production. As in case of native CBP microorganisms, the main

Table 2
Bioethanol production by acid hydrolysis and fermentation of tubers of Jerusalem artichoke.

Type of acid	Sugar (%)	Microorganism/s used	Fermentation conditions	System used	Bioethanol yield (%)	Reference/s
HCl (0.5 N)	20.5	<i>Saccharomyces cerevisiae</i>	37 °C, 24 h, Static-flask fermentation	B	2.76	Kim and Hamdy, 1986
HCl (2%)	15.6	<i>Saccharomyces cerevisiae</i>	30 °C, 72 h, 100 rpm, Shake-flask fermentation	B	1.52 ^a	Raznowski et al. 2011
H ₂ SO ₄ (2%)	15.4	<i>Aspergillus niger</i> co-immobilized with <i>Zymomonas mobilis</i>	35 °C, 14 Days	C	55.1 ^a	Kim and Rhee 1990
NaOH (2%) & H ₂ SO ₄ (4%)	30.0	<i>Saccharomyces cerevisiae</i>	50 °C, 60 h, 150 rpm, Shake-flask fermentation	B	5.56	Li et al. 2016
H ₂ SO ₄ (2%)	97.0	<i>Saccharomyces cerevisiae</i>	30 °C, 72 h, Static-flask fermentation	B	8.2	Szambelan et al. 2004
	97.0	<i>S. cerevisiae</i>	30 °C, 72 h, Static-flask fermentation	B	7.4	Szambelan et al. 2004
	96.0	<i>Zymomonas mobilis</i>	30 °C, 72 h, Static-flask fermentation	B	9.1	Szambelan et al. 2004
	97.0	<i>Z. mobilis</i>	30 °C, 72 h, Static-flask fermentation	B	8.2	Szambelan et al. 2004
	3.3	<i>Z. mobilis</i>	30 °C, 60 h, Static-flask fermentation	B	0.58 ^a	Onsoy et al., 2007

^aYield in g/L/h; B: Batch system; C: Continuous system

Table 3
Bioethanol production by enzymatic hydrolysis and fermentation of inulin-rich feedstocks.

Inulinase source	Inulin-rich feedstock	Sugar (%)	Microorganism/s used	Fermentation conditions	System used	Bioethanol yield (%)	Reference/s
<i>Pichia pastoris</i>	Jerusalem artichoke tubers powder	20.0	Recombinant <i>Saccharomyces cerevisiae</i>	28 °C, 144 h, Static-flask fermentation	B	12.1	Zhang et al. 2010a
<i>Aspergillus niger</i>	Agave neomexicana tubers extract	29.8	<i>Saccharomyces cerevisiae</i>	50 °C, 72 h, 100 rpm, Shake-flask fermentation	B	119 ^a	Mielenz et al. 2015
	Jerusalem artichoke tubers powder	97.0	Co-culture of <i>Kluyveromyces fragilis</i> & <i>Zymomonas mobilis</i>	30 °C, 72 h, Static-flask fermentation	B	9.9	Szambelan et al. 2004
		96.0	Co-culture of <i>Kluyveromyces fragilis</i> & <i>Zymomonas mobilis</i>	30 °C, 72 h, Static-flask fermentation	B	9.4	Szambelan et al. 2004
		99.0	Co-culture of <i>Saccharomyces cerevisiae</i> & <i>Kluyveromyces fragilis</i>	30 °C, 72 h, Static-flask fermentation	B	9.4	Szambelan et al. 2004
		99.0	Co-culture of <i>Saccharomyces cerevisiae</i> & <i>Kluyveromyces fragilis</i>	30 °C, 72 h, Static-flask fermentation	B	8.8	Szambelan et al. 2004
Co-culture of <i>Candida oleophila</i> & <i>Aspergillus niger</i>	Jerusalem artichoke tubers extract	NS	<i>Saccharomyces cerevisiae</i>	30 °C, 72 h, 150 rpm, Shake-flask fermentation	B	1.3	Neagu and Bahrim 2012
	Chicory roots extract	61.0	<i>Saccharomyces cerevisiae</i>	30 °C, 48 h, 120 rpm, Shake-flask fermentation	B	0.73	Abou-Taleb et al. 2019

NS: Not specified; ^aYield in mg/g; B: Batch system

problem is low yield and productivity with prolonged fermentation time. While in engineered microorganisms, major hurdle in the development of industrial CBP microorganism is to achieve high biocatalyst production rate without compromising ethanol productivity. There are few problems associated with the co-expression of multiple genes other than those of interest, tolerance to the culture medium and high production cost in the development of such type of microorganisms. Besides, an extensive investigations must be carried out to check the efficiency of different pretreatment methods on inulin yield and sugar composition of inulin-rich feedstocks. Analysis of different bioprocessing techniques concludes that each process has its pros and cons. Therefore, assessing these bioprocessing strategies straight through the test data is not accurate. Until now, a cost-effective and environmentally benign pretreatment method that can completely saccharify inulin-rich substrates is yet to be established. Moreover, the reaction mechanisms of the different pretreatment technologies have not been explored in detail to improve the existing methods.

4. Biofuels from inulin-rich feedstocks

Ethanol, butanol and acetone are the major biofuels of industrial importance which are produced from corn starch and lignocellulosic waste (straw, bagasse, etc). However, processing/pre-treatment of these raw materials is a cumbersome and lengthy process. Additionally, the requirement of a cascade of enzymes, viz. α -amylase and glucoamylase for the saccharification of starch make it an uneconomical and complex process. In contrast to this, saccharification of inulin enriched materials is an easy, time saving and an economical viable process. A wide variety of inulin-rich materials like chicory root tubers, asparagus root tubers, jerusalem artichoke root tubers, dahlia tubers, etc. can be explored for biofuels production.

4.1. Bioethanol

Bioethanol is a lucrative biofuel with a promising potential for both environmental safety and energy security over fossil fuels. It is produced from a number of substrates like lignocellulosic raw materials, molasses, etc. Different inulin enriched substrates like dahlia, jerusalem artichoke, chicory, asparagus, etc. can also be used for the preparation of bioethanol. These inulin-rich materials are ubiquitously available and are potential renewable energy source for bioethanol production. Different strategies can be employed to produce bioethanol from inulin enriched substrates.

Acidic (HCl and H₂SO₄) hydrolysis of jerusalem artichoke tubers into fermentable sugars have been reported by various investigators (Table 2). In majority of the studies after acidic hydrolysis of inulin-rich materials, *Saccharomyces cerevisiae* (Kim and Hamdy, 1986; Razmovski et al., 2011; Li et al., 2016; Szambelan et al., 2004) and *Zymomonas mobilis* (Szambelan et al., 2004; Onsoy et al., 2007) have been used for the production of biofuels. Inulinases from *Pichia pastoris* (Zhang et al., 2010b) and *Aspergillus niger* (Szambelan et al., 2004; Neagu and Bahrim, 2012; Mielenz et al., 2015) are widely utilized for enzymatic hydrolysis of different inulin enriched substrates, viz. *Agave tequilana* tubers, jerusalem artichoke tubers and chicory roots (Table 3). After hydrolysis, either a single organism (Mielenz et al., 2015; Neagu and Bahrim, 2012) or a co-culture of microorganisms (Szambelan et al., 2004) can be used for the production of biofuels. In a few cases, genetically modified *Saccharomyces cerevisiae* has also been explored to produce bioethanol from tubers of jerusalem artichoke (Zhang et al., 2010a).

In SSF, both hydrolysis and fermentation steps proceed simultaneously in a single vessel for bioethanol production. SSF has been exploited to produce bioethanol from numerous inulin-rich materials (Table 4). Different microorganisms like *Saccharomyces cerevisiae* (Duvnjak et al., 1991; Chang et al., 2008; Matias et al., 2015; Li et al., 2016; Song et al., 2017), *Kluyveromyces marxianus* (Bajpai and Margaritis, 1986; Yuan et al., 2008; Kim et al., 2013) and *Kluyveromyces cicerisporus* (Yu et al., 2010; Yuan et al., 2010) have been utilized to produce bioethanol from jerusalem artichoke tubers in a batch system. In a few cases, a consortium of microorganisms has also been used for bioethanol production. Both *Candida oleophila* and *Aspergillus niger* in a mix culture was used for bioethanol production from chicory roots using a batch system (Abou-Taleb et al., 2019). Similarly, a co-culture of *Saccharomyces* sp. and *A. niger* was exploited to produce bioethanol from jerusalem artichoke tubers using a batch system (Neagu and Bahrim, 2012). To control the issue of feedback inhibition during batch systems, fed-batch system has also been investigated to produce bioethanol from inulin enriched materials. Jerusalem artichoke tubers and stalk extract have been used to produce bioethanol by *Kluyveromyces marxianus* in a stirred tank reactor using fed-batch system (Kim et al., 2013). *A. niger* and *Saccharomyces* sp. co-culture have also been reported to produce bioethanol from jerusalem artichoke tubers powder by static-flask fermentations using a fed-batch system (Ge and Zhang, 2005). Contrarily, a very few reports are existed on ethanol production in continuous mode from inulin enriched feedstocks. Margaritis & Bajpai (1982a) used a continuous system for ethanol production from jerusalem artichoke tubers extract by *Kluyveromyces marxianus* whole cells in a stirred tank

Table 4
Bioethanol production by simultaneous saccharification and fermentation of inulin-rich feedstocks.

Inulin/inulin-rich feedstock	Sugar (%)	Microorganism/s used	SSF conditions	System used	Bioethanol yield (%)	Reference/s
Chicory roots extract	61.0	Co-culture of <i>Candida oleophila</i> & <i>Aspergillus niger</i>	30 °C, 48 h, 120 rpm, Shake-flask fermentation	B	1.56	Abou-Taleb et al. 2019
Coffee waste	4.8	Recombinant <i>Kluyveromyces marxianus</i>	30 °C, 96 h, 100 rpm, Shake-flask fermentation	B	1.0	Galindo-Leva et al. 2016
Jerusalem artichoke tubers extract	NS	<i>Saccharomyces cerevisiae</i>	33 °C, 120 h, Static-flask fermentation	B	0.28 ^a	Duvnjak et al. 1991
	NS		28 °C, 24 h, Static-flask fermentation	B	8.2	Chang et al. 2008
	NS		NS	C	9.0	Chang et al. 2008
	84.5		30 °C, Static-flask fermentation	B	0.45 ^a	Matias et al. 2015
	NS	Co-culture of <i>Saccharomyces</i> sp. & <i>Aspergillus niger</i>	25 °C, 96 h, Static-flask fermentation	B	28.1	Neagu and Bahrim 2012
	NS	<i>Kluyveromyces marxianus</i>	35 °C, 200 rpm, Stirred tank reactor (1L)	C	7.0 ^b	Margaritis and Bajpai 1982a
	NS		35 °C, 12 Days, Borosilicate glass column (1.8 cm × 27 cm)	C	104 ^b	Margaritis and Bajpai 1982b
	NS		35 °C, 7 h, Borosilicate glass column (2 cm × 28 cm)	B	9.2	Bajpai and Margaritis 1986
	69.22		35 °C, 60 h, Static-flask fermentation	B	60.9	Yuan et al. 2008
	NS	Recombinant <i>Saccharomyces cerevisiae</i>	28 °C, 120 h, Stirred tank reactor (5 L)	B	12.6	Wang et al. 2011
Pure inulin from chicory root tubers	NS	Co-culture of <i>Saccharomyces</i> sp. & <i>Aspergillus niger</i>	25 °C, 168 h, Static-flask fermentation	B	15.0	Neagu and Bahrim 2012
	30	Recombinant <i>Saccharomyces cerevisiae</i>	30 °C, 48 h, Static-flask fermentation	B	12.6	Liu et al. 2014
	30		30 °C, 48 h, Static-flask fermentation	B	12.2	Liu et al. 2014
	30		28 °C, 120 h, Stirred tank reactor (5 L)	B	14.7	Wang et al. 2011
	30		28 °C, 96 h, 250 rpm, Stirred tank reactor (2L)	B	14.9	Zhang et al. 2010b
	25		28 °C, 168 h, Static-flask fermentation	B	12.5	Zhang et al. 2015
Pure inulin from Agave root tubers	18.82	Recombinant <i>Kluyveromyces marxianus</i>	30 °C, 36 h, 200 rpm, Shake-flask fermentation	B	8.0	Hong et al. 2015
Jerusalem artichoke tubers powder	NS	<i>Kluyveromyces cicerisporus</i>	25 °C, 48 h, Static-flask fermentation	B	96.3	Yuan et al. 2010
	NS		25 °C, 144 h, 300 rpm, Shake-flask fermentation	B	12.3	Yu et al. 2010
	38.6	Co-culture of <i>Saccharomyces</i> sp. & <i>Aspergillus niger</i>	30 °C, 48 h, Static-flask fermentation	FB	19.6	Ge and Zhang 2005
	5.64	<i>Saccharomyces cerevisiae</i>	30 °C, 12 h, Static-flask fermentation	B	13.8 ^c	Song et al. 2017
	NS		30 °C, 60 h, 150 rpm, Shake-flask fermentation	B	5.5	Li et al. 2016
	NS	Recombinant <i>Saccharomyces cerevisiae</i>	34 °C, 72 h, 180 rpm, Stirred tank reactor (5 L)	FB	8.4	Wang et al. 2015
	30		30 °C, 120 h, Stirred tank reactor (3 L)	B	13.6	Li et al. 2013b
Jerusalem artichoke tubers and stalk extract	NS	<i>Kluyveromyces marxianus</i>	35 °C, 27 h, 200 rpm, Stirred tank reactor (5 L)	B	2.9	Kim et al. 2013
	NS		35 °C, 76 h, 200 rpm, Stirred tank reactor (5 L)	FB	7.0	Kim et al. 2013

SSF: Simultaneous saccharification and fermentation; NS: Not specified; ^aYield in g/g; ^bYield in g/L/h; ^cYield in mL/L; B: Batch system; FB: Fed-batch system; C: Continuous system.

reactor (1L) at 35 °C, under agitation (200 rpm). In another case, immobilized *Kluyveromyces marxianus* whole cells have been used in a continuous system to produce bioethanol from jerusalem artichoke tubers using a borosilicate glass column (1.8 cm × 27 cm, 1 L) at 35 °C for 12 days (Margaritis & Bajpai, 1982b). The operational stability of immobilized biocatalyst onto a suitable support/matrix is a major factor responsible for its long lasting use in a continuous system. Genetically modified microbes supporting high productivity and wide-range pH and temperature stability can also be utilized to produce bioethanol from inulin enriched materials. Recombinant *Kluyveromyces marxianus* (Galindo-Leva et al., 2016; Hong et al., 2015) and *Saccharomyces cerevisiae* (Wang et al., 2011; Liu et al., 2014) have also been used to produce bioethanol by SSF of extracts agave root tubers and jerusalem artichoke tubers, respectively in a batch system. Bioethanol production from jerusalem artichoke tubers powder have also been reported by

genetically modified *Saccharomyces cerevisiae* using a fed-batch system (Wang et al., 2015). Chicory inulin contains a large percentage of long chains of fructose which has a wide range of applications (Gunnarsson et al., 2014). However, finding a suitable microorganism with high conversion efficiency within a short span of time is the major challenge in the bioprocessing of chicory root tubers into bioethanol. Whereas, a medium chain length of inulin present in jerusalem artichoke tubers makes it more suitable for the conversion into high value added products including bioethanol and biochemicals (Li et al., 2013a). Additionally, the presence of biotin and nitrogen in the jerusalem artichoke tubers again justify its potential for direct utilization into a targeted product without adding other nutrients. Although, a plenty of research has been carried out to utilize jerusalem artichoke tubers as a potent feedstock to produce many bio-products, but most of them are derived in a low amount. So, more microorganisms with enhanced capacity for

Table 5
Bioethanol production by consolidated bioprocessing of inulin-rich feedstocks.

Inulin-rich feedstock	Sugar (%)	Microorganism/s used	CBP conditions	System used	Bioethanol yield (%)	Reference/s
Jerusalem artichoke tubers powder	18.9	<i>Kluyveromyces marxianus</i>	37 °C, 24 h, 100 rpm, Jar fermenter (2 L)	B	9.4	Charoensopharat et al. 2015
	NS	<i>Saccharomyces cerevisiae</i>	40 °C, 48 h, Static-flask fermentation	B	7.3	Hu et al. 2012
	NS	Recombinant <i>Saccharomyces cerevisiae</i>	40 °C, 48 h, Static-flask fermentation	B	6.5	Hu et al. 2012
Jerusalem artichoke tubers extract	NS	<i>Saccharomyces cerevisiae</i>	40 °C, 72 h, 150 rpm, Shake-flask fermentation	FB	8.5	Khatun et al. 2017
	NS	<i>Saccharomyces cerevisiae</i>	40 °C, 24 h, Static-flask fermentation	B	3.13 ^a	Wang et al. 2016
	NS	<i>Kluyveromyces marxianus</i>	37 °C, 60 h, Static-flask fermentation	B	9.7	Gao et al. 2015
	55.9	<i>Saccharomyces cerevisiae</i>	30 °C, 34 h, 400 rpm, Stirred tank reactor (5 L)	B	3.6	Lim et al. 2011
	30	Recombinant <i>Saccharomyces cerevisiae</i>	40 °C, 192 h, Stirred tank reactor (5 L)	B	14.0	Wang et al. 2014
	NS	<i>Zygosaccharomyces bailii</i>	40 °C, 84 h, Static-flask fermentation	B	10.2	Du et al. 2014
	NS	<i>Zygosaccharomyces bailii</i>	25 °C, 60 h, Static-flask fermentation	B	3.62 ^a	Paixao et al., 2018

CBP: Consolidated bioprocessing; NS: Not specified; ^aYield in g/L/h; B: Batch system; FB: Fed-batch system

hydrolysis and fermentation step are to be tested for bioethanol production at large scale.

CBP has also been explored to produce bioethanol from inulin enriched materials (Table 5). Numerous microorganisms like *Kluyveromyces marxianus* (Hu et al., 2012; Charoensopharat et al., 2015; Gao et al., 2015), *Saccharomyces cerevisiae* (Lim et al., 2011; Hu et al., 2012) and *Zygosaccharomyces bailii* (Paixão et al., 2018) have been used in this process for bioethanol production from jerusalem artichoke tubers powder/extract using a batch system. In a few cases, genetically modified *Saccharomyces cerevisiae* has also been investigated for bioethanol production in batch (Wang et al., 2014, 2016) and fed-batch (Khatun et al., 2017) systems. The principal challenge in recombinant technology is the heterogeneous expression of inulinases within the recombinant strain which could increase the growth rate and biomass conversion rate. As saccharification of inulin-rich feedstocks is not a cumbersome process, it may led to opening a new era to exploit inulin-rich materials for bioethanol production at large-scale.

4.2. Acetone-butanol

Acetone-butanol are also considered as important biofuels which are mainly produced from molasses and corn starch. The cost of a bioprocess mainly depends upon the type of substrate and complexity of the bioprocess. That is why, it is necessary to identify more economical and ubiquitous substrates for efficient acetone-butanol production. A wide variety of inulin-rich feedstocks like *kazak dandelion*, jerusalem artichoke tubers and chicory tubers has been used for acetone-butanol production (Table 6). *Clostridium acetobutylicum* has been exploited for SSF of jerusalem artichoke tubers extract for acetone-butanol (Marchal et al., 1985) and butanol (Chen et al., 2010) production in a batch system. *Clostridium saccharobutylicum* has also been investigated to produce acetone-butanol-ethanol by SHF of jerusalem artichoke tubers extract (Sarchami and Rehm, 2014, 2015). SSF have also been used for acetone-butanol-ethanol production from *kazak dandelion* extract (Ujor et al., 2015), pure inulin (Ujor et al., 2015) and chicory roots extract (Ujor et al., 2015) using *Clostridium saccharobutylicum* in a batch system.

5. Challenges of lignocellulosic raw materials and inulin-rich feedstocks for biofuels production

Lignocellulosic raw materials are composed of biological polymers such as hemicellulose (25–35%), cellulose (40–50%) and lignin (15–20%), which are associated with each other by strong covalent linkages and hydrogen bonds forming a highly recalcitrant structure. The presence of strong bonds that holds the lignin polymer together renders its solubilization, thereby inhibiting the hydrolysis of cellulose and hemicellulose. So for the utilization of lignocellulosic raw materials for biofuels production, digestibility and availability of cellulosic and hemicellulosic fractions, together with the removal of the lignin fractions must be performed, through a series of targeted pretreatment steps which is a very costly and cumbersome process. However, in case of inulin-rich raw materials the saccharification of inulin enriched substrates is a simple and an efficient process. Moreover, inulin enriched feedstocks grows fast, produces large amounts of biomass, needs relatively few pesticides, fertilizers and water for their growth, and also can be grown on marginal land. That is why they are being considered as a potentially useful substrate for the production of biosolvents, particularly biofuels. The major challenge for the production of biofuels from inulin-rich feedstocks is their availability. Amongst various inulin-rich feedstocks only jerusalem artichoke is cultivated at large scale and it has been explored widely for the production of biofuels. To increase the availability of inulin-rich feedstocks, the cultivation of dahlia, asparagus, chicory, etc. should be promoted in future. Bioprocessing strategies used for the production of biofuels from inulin-rich feedstocks have few limitations which limits their use for biofuels production.

Table 6
Butanol production from inulin-rich feedstocks.

Bioprocess method	Inulin/inulin-rich feedstock	Sugar (%)	Microorganism/s used	Bioprocess conditions	Product	System used	Bioethanol yield (%)	Reference/s
SHF	Jerusalem artichoke tubers extract	0.52 ^a	<i>Clostridium saccharobutylicum</i>	37 °C, 60 h, 200 rpm, Shake-flask fermentation	ABE	B	1.5	Sarchami and Rehmann 2015
		0.52 ^a		37 °C, 60 h, 200 rpm, Shake-flask fermentation	ABE	B	0.25 ^b	Sarchami and Rehmann 2014
SSF	Pure Inulin	2.9	<i>Clostridium saccharobutylicum</i>	35 °C, 96 h, Static-flask fermentation	ABE	B	9.7	Ujor et al. 2015
	Kazak dandelion extract	4.0	<i>Clostridium saccharobutylicum</i>	35 °C, 60 h, Static-flask fermentation	ABE	B	8.5	Ujor et al. 2015
	Chicory roots extract	4.0	<i>Clostridium saccharobutylicum</i>	35 °C, 60 h, Static-flask fermentation	ABE	B	12.5	Ujor et al. 2015
	Jerusalem artichoke tubers extract	NS	<i>Clostridium acetobutylicum</i>	35 °C, 32 h, Static-flask fermentation	Acetone-butanol	B	2.3–2.4	Marchal et al. 1985
				35 °C, 60 h, Static-flask fermentation	Butanol	B	1.1	Chen et al. 2010

SHF: Separate hydrolysis and fermentation; SSF: Simultaneous saccharification and fermentation; ABE: Acetone butanol ethanol; NS: Not specified; ^aYield in g/g; ^bYield in g/L/h; B: Batch system.

Appropriate strategies should be developed to overcome these limitations. Most of them have been used for biofuels production at laboratory scale. So, in future efforts should be made to make trials at large scale for biofuels production from inulin-rich feedstocks.

6. Conclusions

Inulin-rich feedstocks are the robust renewable energy source for biofuels production. Jerusalem artichoke is cultivated widely in different countries. Most of the inulin enriched feedstocks are explored at bench scale for biofuels production, so attempts should be made to observe their techno-economic feasibility at large-scale. The utilization of inulin-rich materials directly in bioprocesses is also a major challenge for biofuels production. Therefore, efforts should be made to develop an efficient CBP for the production of biofuels. Additionally, recombinant species should be developed having good efficiency for CBP of inulin enriched materials to produce biofuels.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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