Agronomy Research **17**(3), 833–849, 2019
https://doi.org/10.15159/AR.19.039

Single cell oil production from waste biomass: review of applicable agricultural by-products

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Abstract. Single cell oil (SCO) is an attractive alternative source of oils, since it can be used as feedstock in biofuel production and also have been recognized as viable option in production of essential fatty acids suitable for either human nutrition or as supplementary in animal feeds. However, the usability of SCO is limited due to the high price of raw materials used in the fermentation process. This problem can be tackled by using low-cost agro-industrial residues which are applicable for SCO production. Use of these by-products as the main carbon source in fermentations not only significantly reduces the overall production costs of SCO, but also enables treatment of generated waste streams, thus reducing the negative impact on environment. Since various biodegradable agro-industrial by-products can be used in microbial fermentations, this review aims to categorize and compare applicable agricultural residues by their availability, necessary pre-fermentation treatments, SCO yields and current usability in other competing sectors.

Key words: microbial oil, oleaginous microorganisms, low-cost substrate, agricultural residues, animal feed, biodiesel.

INTRODUCTION

In order to reduce the costs associated with waste recycling, it is important to look at the possibility of using available waste streams as raw materials for the production of new value added products. Nowadays, a large part of biodegradable waste is incinerated (Johnson & Taconi, 2007) or used as feedstock in biogas (Kost et al., 2013), bioenergy or biofuel (Browne et al., 2011) production, which are products with relatively low added value (Spalvins et al., 2018a). However, thanks to new technological solutions, the same waste products can be used as raw materials for production of products with high added value (Werpy & Petersen 2005; FitzPatrick et al., 2010; El-Bakry et al., 2015; Finco et al., 2016). One such product is single-cell oil (SCO).

Single cell oils are oils derived from oleaginous fungi, yeasts, bacteria, microscopic algae and protists. These oils can be used for animal and human consumption, in pharmaceuticals and as feedstock in production of biofuels (Ratledge, 2013). The chemical and biochemical properties of these oils are similar to those derived from plants and animals (Ward & Singh, 2005; Ratledge & Cohen, 2008; Meng et al., 2009;

Dewapriya & Kim, 2014), however, SCO advantages include the high diversity of applicable oleaginous microorganism species, the ability to accumulate large amounts of lipids in cells, faster growth of biomass compared to plants and animals, and reduced production costs (Huang et al., 2013; Thevenieau & Nicaud, 2013; Garay et al., 2014). In addition, SCO production is a good alternative to plant-derived oils because the production of SCO is more environmentally friendly (Tilman, 1999), consumes less water (Mekannen & Howkstra, 2014), production requires significantly smaller land areas and has a significantly lower negative impact on climate change (Vermeulen et al., 2012), as it is in the case with oils derived from plants or animals (Spalvins & Blumberga, 2018). Another advantage of SCO is the ability to use a wide range of biodegradable agricultural by-products in the cultivations of SCO-producing microorganisms. SCOs used in nutritional supplements, baby foods and pharmaceuticals are produced in microbiological fermentations where refined sugars are used as the main carbon source. Given that the carbon source accounts for 60-75% of the total cost of SCO production (Finco et al., 2016), the total cost of production is considerably increased by the use of refined sugars in fermentations. The increase in costs of using refined sugars makes SCO production not profitable in sectors with relatively lower added value, such as animal feeds and biofuels. Consequently, these sectors would need to use cheaper substrates for SCO production, such as by-products from other industries, waste products, wastewaters and production residues (Spalvins & Blumberga, 2018). The use of waste products in SCO production reduces total oil production costs and waste treatment reduces the negative environmental impact that these wastes would have if they were discharged untreated (Spalvins et al., 2018b).

In the context of this review, agricultural waste is any biodegradable by-product from agriculture or food production industries that is suitable for cultivation of SCO-producing microorganisms and is not further utilized in the relevant production systems or their use in SCO production would lead to higher added value than already existing solutions. In other reviews (Leiva-candia et al., 2014; Jin et al., 2015; Finco et al., 2016; Patel et al., 2016; Qin et al., 2017) which summarize reported findings on the use of suitable waste in cultivations of SCO-producing microorganisms, information focuses mainly on the used microorganisms and not so much on the properties of the waste products themselves. However, nowadays, for research and industrial purposes, access to the various strains of microorganisms is relatively simple, but the availability of suitable waste products is very specific to each particular local economy and nearby industries that generate these wastes. In order to facilitate selection of the most appropriate by-products, the wastes in this paper will be categorized and compared according to availability, pre-fermentation treatment, SCO yields and current use in other competing industries. However, it should be emphasized that a full availability analysis for any waste product that is potentially suitable for SCO production needs to be done by taking into account the costs, local availability, transportation and required logistics systems. Performing such analysis is beyond the scope of this review, but further discussion on the topic of complete availability analysis of waste materials is reviewed by Spalvins & Blumberga (2018).

WASTE TYPES

Spalvins et al. (2018a) categorized the most suitable agricultural wastes for single cell protein (SCP) production in 4 groups: monosaccharide and disaccharide rich sources; starch rich sources; structural polysaccharide rich sources; protein or lipid rich sources. These groups will also be used to categorize waste products in this review. Although the waste products described in the previous review (Spalvins et al., 2018a) were reviewed in regard to SCP production, they are also suitable for SCO-producing microorganisms and vice versa. For this reason, previously reviewed waste products such as whey, bran, monosodium glutamate wastewater etc. were not repeatedly described in this review, although they were listed in the summary tables (Table 1, 2, 3, 4) to compare SCO yields. New or additional information in the regard to SCO production was provided for previously described waste products such as molasses, cereal residues, various starchy wastewaters etc.

COMPARISION OF AGRICULTURAL BY-PRODUCTS

Monosaccharides and disaccharides rich sources

Monosaccharides and disaccharides rich waste products can be directly used in microbial fermentations with good SCO yields. Thus, the main advantage of this waste product group is that they do not require pre-treatment or that the pre-treatment is minimal, which in turn significantly reduces the total cost of SCO production.

Molasses

During sugar beet and sugarcane processing, by-products such as molasses, filter mud, bagasse, straw and tops are produced during the sugar production process (FAO, 1987). For every tonne of sugar produced, approximately 320 kg of molasses are generated, which equals about 60 million tonnes of molasses generated in 2017 (FAO, 1987; OECD-FAO, 2018). Depending on the annual yield and market price changes, about 90% of molasses are used in the production of industrial alcohol. Since SCO is a product with higher added value than ethanol (Duncan, 2003; Thompson et al., 2009; Shepherd & Bachis, 2014), fermentation operations adapted to SCO production can compete with alcohol production for molasses as raw material.Molasses usually contain large amounts of fermentable carbohydrates (45–60%) (Ren et al., 2013) and since molasses do not require pre-treatment (Kopsahelis et al., 2007), the use of molasses in the cultivation of SCO-producing microorganisms has been extensively studied (Voss & Steinbüchel, 2001; Gouda et al., 2008; Zhu et al., 2008; Karatay & Dönmez, 2010; Ren et al., 2013). Depending on used extraction methods and plant species molasses usually also contain mineral elements and small amounts of proteins and lipids (Ren et al., 2013). Very high SCO concentrations have been reported by cultivating bacteria *Gordonia sp*. and *Rhodococcus opacus*, when molasses was used as the main source of carbohydrates (Gouda et al., 2008). High cell densities and SCO rich in unsaturated fatty acids were obtained by cultivating microalgae *Schizochytrium sp*. (Ren et al., 2013) (Table 1).

Brewery wastewater

Most brewery wastewaters are generated during the production, packaging, washing and discharge of beer. Breweries in general have very high water consumption for their operations and the total volume of wastewater generated per 1 litre of beer produced in well managed breweries is 2 litres, while in average breweries from 3 to 6 litres of wastewaters are generated per every litre of beer produced (BA, 2017). Considering that in 2017 the global production of beer amounted to 1 900 million hectolitres (FAO, 2009; KHC, 2018), the amount of wastewater generated from the beer industry is huge. Untreated brewing wastewater is characterized by biological oxygen demand (BOD) and chemical oxygen demand (COD) values from 600 to 5,000 ppm and from 1,800 to 5,500 ppm respectively, with pH varying from 3 to 12 depending on the use of cleaning agents and high nitrogen, phosphorus and suspended solids concentrations (BA, 2017). Since brewery wastewaters are not reutilized in other processes, the huge wastewater volumes that are generated in breweries need to be pre-treated, which in turn considerably increase the overall expenses for the brewery, or heavily overloads local water treatment systems, if appropriate pre-treatment is not carried out in the brewery itself. Brewery wastewaters contain cellulose, sugars, amino acids, spent grains, proteins, sludge, wort, yeast suspended solids and beer residues (BA, 2017). If these wastewaters do not contain microorganism growth inhibiting compounds from cleaning agents, then brewery wastewaters are suitable for cultivating SCO-producing microorganisms such as *Rhodococcus opacus* (Schneider et al., 2013), although biomass and accumulated SCO concentrations are relatively low (Table 1). Additional research and finding of more suitable microorganisms for brewery wastewaters is necessary to ensure more efficient treatment of these wastewater and obtain higher SCO yields.

Sugarcane juice

Sugarcane juice is a popular drink in South America and other regions where sugarcane is widely grown (Soccol et al., 2017). Juice itself is considered a cheap source of sugars and variable amount of juice is spilled during squeezing and cannot be used in human consumption. Since sugarcane juice has high monosaccharide and disaccharide content (15% w/w), sugarcane juice is a suitable raw material for SCO production without requiring addition pre-treatment (Soccol et al., 2017). Sugarcane juice as a cheap raw material is one of the few by-products tested in industrial scale pilot fermentation to produce SCO suitable for biofuel production from oleaginous yeast *Rhodosporidium toruloides* (Soccol et al., 2017).

Sweet sorghum juice

Juice from sweet sorghum plant is used in sugar production (Gnansounou et al., 2005; Liang et al., 2010). In 2017, 57 million tons of sweet sorghum was harvested, which is a miniscule amount compared to sugarcane and sugar beet yearly harvests, but since sweet sorghum juice is being evaluated as a raw material for ethanol production, SCO production as a competitive alternative is also being explored (Liang et al., 2010). Using sorghum juice as a raw material to cultivate *Schizochytrium limacinum* Liang et al. (2010) managed to obtain high SCO concentrations, although biomass concentrations in media were relatively low for this microalgae strain (Table 1).

Table 1. Monosaccharaides and disaccharides rich sources. Recent reports of obtained dry cell weight (DCW) (grams per litre of medium) and lipid content (LC) (% of DCW) by using mono and disaccharide rich wastes as substrates for microbial fermentations

Substrate	Microorganisms	DCW (g L^{-1})	LC (%)	References
Sugarcane molasses	*Rhodococcus opacus*	-	93	Gouda et al., 2008
	Gordonia sp.	-	96	”
	Cunninghamella echinulata	12.1	32	Chatzifragkou et al., 2010
	Mortierella isabellina	9.5	54	”
	Trichosporon fermentans	28.1	62.4	Zhu et al., 2008
	Schizochytrium sp.	35.32	41.2	Ren et al., 2013
Molasses	*Candida lipolytica*	-	59.9	Karatay & Dönmez, 2010
	Candida tropicalis	-	46.8	”
	Rhodotorula mucilaginosa	-	69.5	”
Sugar beet molasses and sucrose	*Rhodococcus opacus*	18.4	38.4	Voss & Steinbüchel, 2001
Cheese whey	*Mortierella isabellina*	32.0	25.3	Vamvakaki et al., 2010
	Thamnidium elegans	18.1	3.3	”
	Mucor sp.	21.7	3.2	”
Sweet whey	*Rhodococcus opacus*	-	84	Gouda et al., 2008
Brewery effluents	*Rhodotorula glutinis*	5.22	12.5	Schneider et al., 2013
Sugarcane juice	*Rhodosporidium toruloides*	0.44 g/L/h (pilot scale)		Soccol et al., 2017
Sweet sorghum juice	*Schizochytrium limacinum*	9.4	73.4	Liang et al., 2010

Starch rich sources

Starch-rich wastes, such as cereal and vegetable processing residues and food waste, make up a large part of the biodegradable agricultural and household wastes. Although these waste products are available in large quantities, they need to be hydrolysed before they can be used in microbial cultivations, which in turn increases the total cost of production of SCO. Costs can be reduced by replacing applied mechanical or chemical hydrolysation treatments with enzymatic using amylolytic microorganisms in pre-fermentations (Pleissner et al., 2013; Lau et al., 2014; Johnravindar et al., 2018) or by using enzymatic hydrolysis (Pleissner et al., 2014; Pleissner et al., 2017; Sloth et al., 2017) to digest the starch present in the waste products.

Food waste

Globally food waste constitutes to approximately 1.3 billion tonnes annually (Gustavsson et al., 2011; Pleissner et al., 2013). The composition of food waste varies depending on the source, but usually contains 30–60% carbohydrates (mostly starch), 6–10% protein and 7–30% fat (Leung et al., 2012; Lau et al., 2014; Pleissner et al., 2014), therefore, after appropriate hydrolysis, these residues can be used for SCO production (Pleissner et al., 2013; Lau et al., 2014; Pleissner et al., 2014; Pleissner et al., 2017; Sloth et al., 2017; Johnravindar et al., 2018). Although the amount of generated food waste is huge, a large proportion of these residues is mixed with non-biodegradable residues (other municipal residues), so the availability of real food residues is considerably lower. If local households and catering business chains sort food waste separately and effective collection of these residues is organized, then amounts of locally available food waste can be sufficient for industrial scale SCO production. By using hydrolysed food waste the highest reported SCO yields have been achieved by cultivating oleaginous yeast *Yarrowia lipolytica* (Johnravindar et al., 2018) (Table 2).

Table 2. Starch rich sources. Recent reports of obtained dry cell weight (DCW) (grams per litre of medium) and lipid content (LC) (% of DCW) by using starch rich wastes as substrates for microbial fermentations

Substrate	Microorganisms	DCW ($g\ L^{-1}$)	LC (%)	References
Food waste hydrolysate	*Schizochytrium mangrovei*	14	16	Pleissner et al., 2013
	Chlorella pyrenoidosa	20	20	”
	Galdieria sulphuraria	3.5	-	Sloth et al., 2017
	Chlorella vulgaris	20	35	Lau et al., 2014
	Yarrowia lipolytica	20.9	49.0	Johnravindar et al., 2018
	Rhodotorula glutinis	14	47	
	Cryptococcus curvatus	9.4	29	”
	Chlorella pyrenoidosa	31.7	14.1	Pleissner et al., 2017
Potato processing waste-water	*Aspergillus oryzae*	3.5		Muniraj et al., 2013
Corn steep water and corn gluten water	*Rhodotorula glutinis*	26.4	28.9	Liu et al., 2016
Corn starch wastewater	*Rhodotorula glutinis*	40	35	Xue et al., 2010
Cassava starch hydrolysate	*Rhodosporidium toruloides*	22.0	63.4	Wang et al., 2012
Corn starch hydrolysate and defatted soybean meal	*Mortierella isabellina*	29.5	31.1	Zhu et al., 2003

Potato processing wastewater

Potato is one of the most popular staple foods in many parts of the world, as well as an important source of carbohydrates. Potato processing industries generate large amounts of wastewater during production of potato chips, peeled whole potatoes, potato slices and many other potato products. In 2017, 388 million tons of potatoes (FAO, 2019) were harvested globally and the global potato processing industry generated approximately 30 million tonnes of wastewater (Stevens & Gregory, 1987; Hung et al., 2004). Potato wastewaters are rich in starch and protein and these effluents have high COD (10,000–11,000 ppm), BOD (4,000–6730 ppm) and suspended solids (5,150–18,000 ppm) values (Gray & Ludwig, 1943; Cooley et al., 1964; Hung et al., 2004). Consequently, the potato processing industry poses a risk to local environments if these wastewaters are discharged untreated (Hung et al. 2004). Such technological solutions as screening, sedimentation, flotation, earthen ponds, activated sludge, anaerobic treatment, microstraining, chemical coagulation and many others have been developed and are being actively used for potato processing wastewater treatment. However, the effective use of these wastewaters in microbial fermentations have been scarcely reported (Muniraj et al., 2013) and more research on suitable microorganism strains is needed.

Corn starch processing wastewater

In 2017, global corn starch production was 70 million tonnes, resulting in more than double the amount of wastewater (Xue et al., 2010; FAO, 2019). Because corn starch wastewater is rich in starch, its release into natural water bodies can cause environmental pollution (Lu et al., 2009). However, due to the high carbohydrate content, corn starch wastewater can be used in microbial fermentations (Xue et al., 2010). By using this wastewater in SCO production, its suitability has been studied using it either as a carbon source (Xue et al., 2010) or as a nitrogen source (Liu et al., 2016) in cultivations of oleaginous yeast *Rhodotorula glutinis* (Table 2).

Low cost products

Although not waste products, low cost substrates such as cassava and corn starch and soybean meal are widely used in industrial fermentation processes, where starch compounds are used as carbon source and soybean residues are used as nitrogen source (Wang et al., 2012; Zhu et al., 2003). By using *Rhodosporidium toruloides* on cassava starch high biomass and SCO concentrations have been reported (Wang et al., 2012). By using corn starch hydrolysate and defatted soybean meal as combined substrate, very high biomass concentrations have been reported (Zhu et al., 2003), although lipid content could be higher considering that SCO concentrations as high as 65% have been reported in *Mortierella isabellina* biomass (Fakas et al., 2009) (Table 2).

Structural polysaccharide rich sources

Cereals residues

Wheat, maize and rice make up 43% of the world's food calories (FAO, 2019). Cereals are the most widely grown agricultural crop and during the processing of cereals a huge amount of residues are generated, which are widely available (Spalvins et al., 2018a). Due to the vast amounts, the use of cereal residues in the cultivation of microorganisms is of great economic and ecological importance. Cereal processing residues are rich in lignocellulose, therefore, the use of these residues as a carbon source for microbiological fermentations is much more complicated because of the need for extensive mechanical, chemical or biochemical pre-treatment, which increase the overall production costs. Additionally, during pre-treatment, hydrolysates release microorganism growth inhibiting compounds such as furfural, vanillin, phydroxybenzaldehyde, syringaldehyde, and others (Yu et al., 2014), therefore, hydrolysates need to be diluted or detoxified, which further complicates the use of these materials in SCO production. Despite these challenges, the use of cereal residues in SCO production has been extensively studied, and researchers have managed to obtain high concentrations of microbial biomass and SCO in mediums derived from cereal residues such as straw, stover, corncob residues and grain hulls (Zhu et al., 2003; Gouda et al., 2008; Huang et al., 2009; Huang et al., 2011; Yu et al., 2011; Galafassi et al., 2012; Huang et al., 2012a; Ruan et al., 2012; Gao et al., 2014; Yu et al., 2014; Chang et al., 2015; Kahr et al., 2015; Poontawee et al., 2017; Guerfali et al., 2018). The highest SCO yields for cereal residue substrates were reported using the yeasts form *Trichosporon* genus - *Trichosporon cutaneum* (Gao et al., 2014), *Trichosporon fermentans* (Huang et al., 2009) and Trichosporon dermatis (Huang et al., 2012a) (Table 3).

Fruit and vegetable waste

The composition of the fruit processing residues depends on which parts of the fruit or plant make up most of the generated waste. Waste products such as date fruit and tree residues have been extensively studied for their use in various microbiological fermentations (Chandrasekaran & Bahkali, 2013), however, the use of these waste products in SCO production requires further studies (Gouda et al., 2008). In 2017, global production of date fruit amounted to 8 million tonnes (FAO, 2019) of which at least 10% end up as waste in the form of date pits and spoiled date fruits (Chandrasekaran & Bahkali, 2013).

Tomato processing industry generates a large amount of residues during peeling of tomatoes. Approximately 10–40% of the total volume of the processed tomatoes ends up as waste. Considering that around 70% of all produced tomatoes are processed (Strati & Oreopoulou, 2011), about 32 million tonnes of tomato residues are generated each year (FAO, 2019). Tomato residues are rich in lignocellulose, proteins and lipids, and these residues are also good source of vitamins and mineral elements (Al-wandawi et al., 1985). Thus, if these residues cannot be used in animal nutrition due to transportation, these residues could be used as rich medium for SCO-producing microorganisms. Results reported so far using *Cunninghamella echinulata* (Fakas et al., 2008), *Rhodococcus opacus*, *Gordonia sp*. (Gouda et al., 2008), shows that tomato residues are an effective substrate for SCO production (Table 3).

Sugar processing waste

The main fibre-rich sugar processing residues are bagasse and straw. During processing, approximately 250 kg of bagasse and 60 kg of straw are generated from one tonne of processed sugarcane, resulting in an annual production of around 460 million tonnes of bagasse and 110 million tonnes of straw (FAO, 1987; OECD-FAO, 2018; FAO, 2019). Bagasse is widely used as a fuel and as raw material for biofuel production or paper production (Hofsetz & Silva, 2012). Since the amount of fibre-rich residues generated by the sugar processing industry is huge, more efficient uses and the production of products with higher added value using sugar processing residues have been extensively studied (Tsigie et al., 2011; Huang, et al., 2012b; Bonturi et al., 2017; Unrean et al., 2017; Poontawee et al., 2018). The highest SCO yields using begase hydrolysates have been reported using yeast *Trichosporon fermentans* (Huang et al., 2012b) (Table 3).

Sugarcane bagasse hydrolysate is characterized by relatively low C/N ratios due to its high concentration of proteins and other nitrogen compounds (Bonturi et al., 2017). Hence, these hydrolysates require additional carbon sources to increase the C/N ratio in the medium and promote oil accumulation in the microorganisms.

Table 3. Structural polysaccharides rich sources (agricultural waste). Recent reports of obtained dry cell weight (DCW) (grams per litre of medium) and lipid content (LC) (% of DCW) by using structural polysaccharides rich wastes as substrates for microbial fermentations

Substrate	Microorganisms	DCW ($g\ L^{-1}$)	LC (%)	References
Wheat straw hydrolysate	*Cryptococcus curvatus*	17.2	33.5	Yu et al., 2011
	Rhodotorula glutinis	13.8	25.0	”
	Rhodosporidium toruloides	9.9	24.6	”
	Lipomyces starkeyi	14.7	31.2	”
	Yarrowia lipolytica	7.8	4.6	”
Rice straw hydrolysate	*Trichosporon fermentans*	28.6	40.1	Huang et al., 2009
Corn stover hydrolysate	*Rhodotorula graminis*	-	34	Galafassi et al., 2012
	Trichosporon cutaneum	17.35	23.5	Huang et al., 2011
	Mortierella isabellina	14.08	34.5	Ruan et al., 2012
Corncob hydrolysate	*Trichosporon cutaneum*	38.4	32	Gao et al., 2014
	Cryptococcus sp.	12.6	60.2	Chang et al., 2015
	Trichosporon dermatis	24.4	40.1	Huang et al., 2012a
	Yarrowia lipolytica	16.6	19.4	Kahr et al., 2015
Rice hulls hydrolysate	*Mortierella isabellina*	-	64.3	Economou et al., 2011
Wheat bran	*Rhodococcus opacus*	-	56	Gouda et al., 2008
	Gordonia sp.	-	41	”
Barley hull hydrolysate	*Trichosporon cutaneum*	17.5	38.2	Guerfali et al., 2018
Orange waste	*Gordonia sp.*	1.88	80	Gouda et al., 2008
Apple pomace	*Rhodococcus opacus*	-	83	”
	Gordonia sp.	-	70	”
Date waste	*Rhodococcus opacus*	-	57	”
	Gordonia sp.	-	61	”
Tomato waste hydrolysate	*Cunninghamella echinulata*	11.8	48	Fakas et al., 2008
Tomato peel waste	*Rhodococcus opacus*	-	73	Gouda et al., 2008
	Gordonia sp.	-	52	”
Sugarcane bagasse hydrolysate	*Trichosporon fermentans*	31	39.9	Huang et al., 2012b
	Rhodosporidium toruloides	19.0	53.6	Bonturi et al., 2017
	Yarrowia lipolytica	13.7	78.5	Unrean et al., 2017
	Yarrowia lipolytica	11.42	58.5	Tsigie et al., 2011
Sugarcane top hydro-lysate and crude glycerol	*Rhodosporidiobolus fluvialis*	24.3	75.0	Poontawee et al., 2018
Olive mill waste	*Rhodococcus opacus*	-	20	Gouda et al., 2008
	Gordonia sp.	-	29	”
Olive oil wastewater	*Lipomyces starkeyi*	0.054	29.5	Yousuf et al., 2010

Olive processing waste

Olive processing and olive oil production is an important source of income for local economies in the Mediterranean region. Depending on the used milling process, from

500 to 1500 litres of wastewaters are generated from each ton of processed olives. Olive processing wastewaters are rich in carbohydrates, polysaccharides, polyphenols, nitrogen compounds and polyalcohols (Canepa et al., 1988). These wastewaters have very high COD (around 100,000 ppm) and BOD (around 40,000 ppm) values, therefore they need to be treated. Often, these wastewaters are discharged on fields, in lakes, rivers and seas or stored in evaporation lagoons. Such disposal of these wastewaters cause pollution of soil, local water bodies, groundwater and also cause odour pollution (Canepa et al., 1988). Currently, the most effective solutions for olive processing wastewater treatment are biological treatment using anaerobic, aerobic and co-digestion processing techniques. Since olive processing wastewater contains phenolic compounds which inhibit microbial growth, it is necessary to dilute these wastewaters so that they can be used effectively in SCO production (Yousuf et al., 2010). However, even after diluting these wastewaters, the resulting SCO and biomass concentrations have been low (Gouda et al., 2008; Yousuf et al., 2010) (Table 3).

Protein or lipid rich sources

Oils and fats can be used as a carbon source in microbiological fermentations if the used microorganism strain is capable of utilizing these lipids (Fickers et al., 2005). For efficient use of waste oils and fats, mechanical (ultrasonic homogenisation, high-shear emulsifiers, etc.) or chemical (various polysorbates) emulsification solutions are needed during the preparation of the media. Although lipid emulsification is not as costly pre-treatment as the polymer hydrolysation, it still increases the overall cost of production when compared to monosaccharide and disaccharide-rich sources.

In addition to the carbon source, microorganisms require nitrogen, amino acids, fatty acids and micro and macro elements to ensure optimal growth and production of SCO. To break down fibrous proteins in the waste and use them as a source of nitrogen and amino acids in fermentations, these protein compounds need to be hydrolysed using physical, chemical or biochemical pre-treatment techniques (Atalo & Gashe, 1993).

Waste cooking oil

Cooking oils are widely used throughout the world for food preparation in households, canteens and also at industrial scale. During cooking, harmful compounds such as lipid peroxidation products, aldehydes, etc. are released in oils (Wei et al., 2011). Therefore, it is necessary to change the cooking oil regularly and dispose of the waste cooking oils (WCO). Every year, more than 10 million tonnes of WCO are generated globally (Wei et al. 2011). These oils are two to three times cheaper than vegetable oils, therefore, their use in both biodiesel production and as ingredient in animal feeds offers significant economic benefits (Phan & Phan 2008; Talebian-Kiakalaieh et al., 2013). However, since 2002, the European Union has banned the use of WCO in animal feed, as there is a risk that harmful compounds present in oils may be carried over to animal products (Cvengros & Cvengrosova, 2004; Kulkarni & Dalai, 2006). As a result, the main use of spent cooking oils remains in the production of biodiesel, which, compared to SCO production, is a solution with lower added value (Lipinsky, 1981; Browne et al., 2011; Spalvins et al., 2018a). The use of WCO in the cultivation of microorganisms is problematic as it is necessary to emulsify the oils in the prepared media using either chemical emulsifying agents or mechanical emulsification solutions (Michely et al., 2013). An interesting approach to the use of WCO in the cultivation of microorganisms is by using microorganism strains that themselves produce oil emulsifying compounds such as oleaginous yeast *Yarrowia lipolytica* (Michely et al. 2013). When WCO were used as carbon source in the fermentations the reported SCO yields have been miniscule (Table 4), thus additional research is needed to find oleaginous microorganisms that can effectively utilize lipids in media.

Table 4. Protein or lipid rich sources. Recent reports of obtained dry cell weight (DCW) (grams per litre of medium) and lipid content (LC) (% of DCW) by using protein or lipid rich wastes as substrates for microbial fermentations

Substrate	Microorganisms	DCW ($g\ L^{-1}$)	LC (%)	References
Olive oil	*Rhodococcus opacus*	0.21	19	Gouda et al., 2008
	Gordonia sp.	0.56	13	”
Sesame oil	*Rhodococcus opacus*	0.45	67	”
	Gordonia sp.	1.21	50	”
Castor oil	*Rhodococcus opacus*	0.38	58	”
	Gordonia sp.	0.41	49	”
Cotton oil	*Rhodococcus opacus*	0.32	38	”
	Gordonia sp.	0.48	50	”
Peanut oil	*Rhodococcus opacus*	0.23	52	”
	Gordonia sp.	0.35	40	”
Maize oil	*Rhodococcus opacus*	0.63	40	”
	Gordonia sp.	0.85	40	”
Sun flower oil	*Rhodococcus opacus*	1.06	44	”
	Gordonia sp.	1.18	52	”
Rapeseed oil	*Yarrowia lipolytica*	-	-	Papanikolaou & Aggelis, 2003
Monosodium glutamate wastewater	*Rhodotorula glutinis*	2.44	9.04	Xue et al., 2006
Monosodium glutamate wastewater	*Rhodotorula glutinis*	25	20	Xue et al., 2008

CONCLUSIONS

In this review, most of the agricultural wastes that can be used in SCO production have been categorized and discussed more closely. Since agricultural waste groups were categorized in the same way as it was done previously for SCP production (Spalvins et al., 2018a), the same advantages and disadvantages can be referred to these wastes as well with few additions.

Monosaccharides and disaccharides rich sources require minimal pre-treatment which give these wastes technological and economic advantages over other waste types. However, these wastes are already widely used in other fermentation processes and as feedstock in animal feeds. Therefore, each waste material must be evaluated in regard to its economic feasibility and compared with already existing or potentially emerging competing sectors.

Use of starch, protein or lipid rich sources and their hydrolysates in SCO production result in comparatively lover SCO yields than if monosaccharaides and disaccharides or fibre-rich materials are used in fermentations. Regardless of this, waste products such as food waste, potato and corn starch processing wastewaters and waste cooking oils are generated in huge amounts each year in all parts of world. In order to reduce the negative environmental impact and improve SCO production efficiency, additional research is needed to develop more efficient methods of waste hydrolysis and medium detoxification.

Structural polysaccharides rich wastes are available in huge quantities all over the world; therefore, using these wastes have limited competition with other industries which use waste as resource for production of other value-added products. These wastes require extensive pre-treatments and during hydrolysation microbial growth inhibiting compounds may be released, which, in turn, require additional detoxification of the substrate, before these wastes can be used in microbial fermentations.

The key considerations for choosing the most suitable waste product for SCO production are similar with the ones concluded in previous reviews (Spalvins et al., 2018a; Spalvins et al., 2018b) with few changes for details specific to SCO production. Key consideration are: target market for the final oil (biodiesel production; animal feeds); which microorganism strain produces necessary fatty acid profile for the target market; local availability of the particular waste product; pre-treatment costs of the waste product before using it in fermentation; the costs of transportation of the waste product; maximum obtainable cell densities in the substrate; SCO concentrations in the final biomass after fermentation; estimation whether cultivation conditions can be efficiently maintained (energy and heat consumption); efficiency of biomass and waste separation, and SCO extraction (oil extraction from biomass and removal of impurities) methods.

In the future, it is also necessary to review and compare different industrial wastes in regard for their use as substrates for SCO production.

ACKNOWLEDGEMENTS. The work has been supported by ERAF project KC-PI-2017/60 'Supercritical Omega-3 oil from production by-products' managed by the Investment and Development Agency of Latvia (LIAA).

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