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Single Cell Oil Production from Waste Biomass: Review of Applicable Industrial By-Products

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Abstract – **Single cell oil (SCO) is an attractive alternative source of oil, which, depending on the fatty acid composition, can be used as a feedstock for biodiesel production, as an ingredient for pharmaceuticals or as a source of essential fatty acids for human and animal consumption. However, the use of SCO is limited due to use of relatively expensive food or feed products in the cultivation of SCO producing microorganisms. In order to reduce SCO production costs, the use of cheaper feedstock such as biodegradable agro-industrial wastes are necessary. At the same time, the microbial treatment of biodegradable wastes ensures the neutralization of environmentally harmful compounds and reduces the negative impact on the environment. Oleaginous microorganisms are capable of fermenting a variety of industrial by-products, waste products and wastewaters, however further discussion on properties of the waste materials is necessary to facilitate the selection of the most appropriate waste materials for SCO production. Thus, this review compares various industrial waste products that can be used as cheap feedstock for the cultivation of SCO producing microorganisms. Industrial waste products, by-products and wastewaters are compared according to their global availability, current use in competing industries, required pre-fermentation treatments, oleaginous microorganism cell concentrations and SCO yields.**

Keywords – **Animal feed; biodiesel; industrial waste; low-cost substrate; microbial oil; oleaginous microorganisms; resource availability**

1. INTRODUCTION

Waste recycling can significantly reduce the negative impact on the environment and, at the same time, reduce the costs associated with waste management. However, most of the waste is still being recycled using low-added value solutions [1] such as incineration [2], biogas production [3], or bioenergy and biofuel production [4]. In order to increase revenue generated by waste management, it is necessary to introduce new technological solutions, which would enable the use of biodegradable waste products in production of high added value products such as building block chemicals [5], [6] single cell proteins [1], [7], enzymes [8] and others. One of these high added value products is single cell oil (SCO).

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Single cell oils are lipids derived from bacteria, fungi, yeasts, microscopic algae and protists. Depending on their fatty acid composition, these lipids have different applications. SCOs with high saturated fatty acid contents are suitable for the biodiesel production [9], while SCOs rich in polyunsaturated fatty acids are suitable for human and animal nutrition as well as for production of pharmaceuticals [10], [11]. These oils are similar in composition to those that can be obtained from plants and animals, but SCO has several advantages over traditional oil sources:

– A wide range of microorganisms can be used for SCO production. So far, hundreds of microorganisms from more than 60 genera have been identified as suitable for SCO production, capable of accumulating at least 20 % oil relative to its biomass dry weight [11]–[13];
– The growth of microorganisms is considerably faster than growth of plants or animals. Microscopic algal populations double within 2–6 hours, yeast and microscopic fungi populations in 1–3 hours and bacterial populations double within 0.5–2 hours. Therefore, microbial reactors require an oil synthesis cycle of 12–72 h for bacteria and 5–10 days for yeast, fungi and algae, while it is possible to harvest agricultural produce only once a season (1–2 times a year);
– Thanks to the rapid growth of microorganisms, suitable strains of microorganisms can be chosen and artificially selected in a few weeks or months, while breeding takes years with plants and animals;
– Microorganisms have several times higher oil content in dry matter than plants and animals (20–80 % for microorganisms, 5–35 % for plants, 2–30 % for fish) [11], [14];
– The composition of SCOs is of higher quality than that of oils derived from plants or fish. For example, the concentration of high-quality omega-3 fatty acids (EPA and DHA) from the total oil content of SCOs can reach up to 40 %, while for plants and fish it is 4.9 % and 3 %, respectively [11];
– Microorganisms can use different sources of carbon for nutrients. Consequently, it is possible to extract oil using different types of biodegradable waste products with high carbon or carbohydrate content, thus significantly reducing production costs;
– Autotrophic microorganisms (microscopic algae, photosynthetic bacteria) are capable of growing using CO_2 as a carbon source. Thanks to the Wood-Ljungdahl biochemical cycle or reverse Krebs cycle, microorganisms are 3 to 10 times more efficient CO_2-absorbers than plants [15], which generally ensures faster biomass growth and reduced negative environmental impacts;
– The cultivation of microorganisms for oil extraction is independent of seasonal weather conditions and climatic changes. The process of cultivation in the reactors is protected from extreme weather conditions, which usually destroy plant crops grown for oil production. Unlike plants, microorganisms that do not require light for growth, can be cultivated around the clock;
– Cultivation of microorganisms consumes considerably less water than cultivation of crops in agricultural areas. Due to the evaporation, transpiration and leakage of water, average water consumption per 1 kg of cereal is an average of 1800 litres [16]. When cultivating microorganisms in bioreactors, none of these factors have any effect on water consumption;
– The cultivation of microorganisms does not require fertile land, so it does not compete with agriculture. Due to low water consumption, SCO production can also be done in dry climate regions where the availability of fertile land is limited.

All of these advantages have served as a basis for increased use of SCO and SCOs are now used in infant formulas, nutritional supplements and production of pharmaceuticals [10], [11]. However, the SCOs used in these industries are produced from microbial fermentations of raw materials such as refined sugars. By using so-called food and feed materials, the total cost of production is considerably increased [11], [13]. This increase in cost makes SCO production unprofitable for sectors with relatively lower added value, such as biofuels and animal feeds. Therefore, in order to be able to use SCO in these sectors, it is necessary to use cheaper raw materials such as biodegradable by-products, waste products and wastewaters from other industries [13], [17].

Industrial waste is any industrial residue that is not further used in the relevant systems. Industrial waste can come from factories, industries, mills and mining operations [13]. Although industrial waste includes residues such as chemical solvents, pigments, dyes, metal processing waste, radioactive waste, etc., only biodegradable industrial wastes such as sludge, paper waste and production residues, specific industrial and chemical by-products and waste gases can be used for microbial fermentation [7]. Spalvins and Blumberga [13] reviewed the most suitable agricultural by-products for SCO production. Within the framework of this review, the reviewed industrial wastes will be residues suitable for the production of SCO, that are not directly related to agriculture or food production. Other reviews which summarize the use of suitable waste products for production of SCO [11], [18]–[22], the main focus is on the used microorganisms and not so much on the properties of the waste products. Thus, in this review, waste products will be categorized, compared and described according to their availability, required pre-fermentation treatments, SCO yields, oleaginous microorganism biomass concentrations and current use in other industries, to facilitate the selection of the most suitable waste products. It is necessary to emphasize that, in order to do a complete availability analysis, each potential waste product needs to analysed by taking into account its costs, local availability, transportation requirements and necessary logistics and infrastructure systems. Carrying out such an analysis for each of the waste products described in the review is beyond the scope of this review, but the subject of the full availability analysis for waste products is further discussed by Spalvins and Blumberga [17].

2. WASTE TYPES

Spalvins et al. [7] categorized the most suitable industrial wastes for single cell protein (SCP) production in 3 groups: polymer-rich sources; carbon compounds; sources for photosynthetic microorganisms. These groups will also be used to categorize waste products in this review. Although the waste products described in the previous paper were reviewed in regard to SCP production [7], they are also suitable for SCO-producing microorganisms and vice versa, since waste products serve mostly as a carbon source in the fermentation medium. For example, yeast *Yarrowia lipolytica* can be cultivated using leaf juice as a substrate, but a paper reporting it [23] focused on using this yeast for production of SCP, not SCO. In this example it does not matter if leaf juice is used as a carbon source, since the difference between turning *Yarrowia lipolytica* into SCP or SCO producing unit is the difference between C/N ratios, while the source of carbon is irrelevant. For this reason, previously reviewed waste products such as paper waste, acetic acid and hydrocarbons were not repeatedly described in this review, although they were listed in the summary table (Table 1) to compare SCO yields. New or additional information in regard to SCO production was provided for previously described waste products such as glycerol and sewage sludge.

3. Comparison of Industrial By-Products

TABLE 1. INDUSTRIAL WASTES APPLICABLE FOR SCO PRODUCTION

Polymer-rich sources	**Microorganisms**	**DCW, g/l**	**LC, %**	**Ref.**
Paper mill sludge	*Cryptococcus vishniaccii*	14.6	53.40	[24]
Waste paper hydrolysate	*Cryptococcus curvatus*	17.6	52.2	[25]
	Cryptococcus curvatus	15.20	37.8	[26]
Sewage sludge	*Lipomyces starkeyi*	9.5	68	[27]
Carbon compounds				
Glycerol	*Cryptococcus curvatus*	118	25	[31]
”	*Schizochytrium sp.*	151.40	52.36	[32]
Glucose and glycerol	*Schizochytrium limacinum*	88.32	83.84	[28]
”	*Schizochytrium limacinum*	34.43	27.62	[29]
”	*Yarrowia lipolytica*	17	38	[35]
”	*Schizochytrium limacinum*	26.4	75	[29]
Crude glycerol	*Schizochytrium limacinum*	11.78	26	[30]
”	*Rhodosporidium babjevae*	9.9	34.9	[33]
”	*Rhodosporidium diobovatum*	14.1	63.7	[33]
”	*Kodamaea ohmeri*	10.50	32.2	[34]
”	*Trichosporonoides spathulata*	10.40	44.5	[34]
”	*Yarrowia lipolytica*	8.1	43	[36]
”	*Yarrowia lipolytica*	4.92	30.1	[37]
”	*Yarrowia lipolytica*	8.1	43	[38]
Sodium gluconate	*Rhodococcus opacus*	–	80	[39]
”	*Gordonia sp.*	–	72	[39]
Butanol wastewater	*Trichosporon dermatis*	7.4	13.5	[40]
Acetic acid	*Cryptococcus curvatus*	–	60	[41]
Tetradecane	*Rhodococcus opacus*	1.89	62	[39]
”	*Gordonia sp.*	2.01	60	[39]
Hexadecane	*Gordonia sp.*	1.89	58	[39]
Dodecane	*Rhodococcus opacus*	0.70	84	[39]
Sources for photosynthetic microorganisms				
Carbon dioxide	*Dunaliella tertiolecta*	–	44	[42]
”	*Chlorella vulgaris*	2	38	[43]

Note: DCW – dry cell weight (grams per litre of medium); LC – lipid content (% of DCW).

3.1. Polymer-Rich Sources

Cellulose, hemicellulose and sludge fibres as waste are accumulated from paper industry, municipal waste and wastewater treatment systems. Polymers, such as lignocellulosic wastes are the most widely available industrial wastes. These polysaccharides require mechanical, chemical or biochemical pre-treatments to break down polymers and make fermentable carbohydrates available to oleaginous microorganisms, which in turn increases the cost of SCO production [7].

3.1.1. Paper Mill Sludge

Paper mill sludge (PMS) is a waste product generated during paper production. Approximately 30–50 kg of PMS is generated per tonne of paper produced [24], [44]. Thus, every year, the global paper industry generates around 17 million tonnes of PMS [7], [45], from which most of it is dumped in landfills, used in land applications or incinerated [46], [47]. Since PMS is available in large amounts and has not been efficiently utilized thus far, its use in SCO production would be an appropriate value-added solution. PMS is rich in organic compounds and micro and macro nutrients [46], [47]. The composition of PMS varies greatly depending on the type of wood used for paper production, the amount and type of used recycled paper, applied production technology, the target product and other factors [46], [47]. In general, the main components in PMS are cellulose, hemicellulose and lignin, but compared to other lignocellulosic waste products, lignin content in PMS is significantly reduced due to the pulping process [24]. Reduced lignin concentrations significantly improve PMS fermentability and alleviate necessity for extensive pre-treatment processes, which are required for other lignocellulosic materials, thus significantly affecting overall cost of production [1], [7], [24]. Due to these properties, pre-treatment methods such as ultrasonication can be used for preparing PMS for microbial fermentation. Ultrasonication ensures break down of cellulose and hemicellulose and release of fermentable carbohydrates [24], [27]. The high cellulose content ensures that hydrolysed PMS is rich in glucose, xylose, arabinose and other fermentable sugars.

Spalvins et al. [7] previously described the availability and use of waste paper for cultivation of SCP-producing microorganisms. Similar to paper waste, PMS is contaminated with unwanted microorganisms and since the aforementioned ultrasonication pre-treatment does not sterilize the material, a further sterilization is required to control microbial contamination. An interesting solution to this problem would be the use SCO-producing extremophiles [48], [49], thus enabling application of selective growth conditions in the PMS medium. Thus far, good SCO yields have been reported by using PMS as substrate in *Cryptococcus vishniaccii* culture. *Cryptococcus vishniaccii* is psychrophilic yeast which is capable of growth at temperature ranges from 4 °C to 26 °C [50], although in previously mentioned report selective temperatures were not used during fermentation. The use of PMS in SCO production has not been extensively studied, but the reported SCO yields indicate that this substrate is promising [24] and additional research on PMS as well as on paper waste in SCO production are required [7], [25], [26].

3.1.2. Sewage Sludge

Sewage sludge is a waste product generated by wastewater treatment plants. In European Union alone about 33 million tonnes of sewage sludge are generated annually [51]–[54]. The use of sewage sludge in land applications is limited due to risk of contaminating soil and water with heavy metals and toxic organic compounds. For these reasons, sewage sludge is mainly disposed in landfills or dried and then burned [27]. Value added and environmentally friendly alternatives to sewage sludge management include: use in biogas production, as well as aerobic fermentation for SCO production, where the produced oil could be used for biofuel production [27]. Another interesting alternative is the use of oleaginous microorganisms directly in aerobic wastewater treatment systems [55]. In this way, the sludge's lipid content would be significantly increased and it would enable the use of the sewage sludge as a direct feedstock for the production of biodiesel, without the need to separately treat sewage sludge afterwards.

Usually, sewage sludge is rich in proteins, carbohydrates, fats, fibres, nitrogen compounds and also contains phosphorus and sulphur compounds and many other micro and macro elements [56], [57]. Sewage sludge have high organic carbon concentrations, which are mainly found in the form of fibres, thus hydrolysis of the sludge can ensure the breakdown of fibres and release of fermentable carbohydrates [27]. Sewage sludge has high nitrogen concentrations and the average C/N ratios are around 6 [56], [57], which means that in order to efficiently use sewage sludge in SCO production, it is necessary to significantly increase carbon concentrations by additionally adding other carbohydrate-rich by-products. Thus far, good SCO yields have been reported by [27] when *Lipomyces starkeyi* was cultivated in treated sewage sludge enriched with glucose to reach appropriate C/N values [27]. Due to the high concentrations of nitrogen, proteins, fatty acids and micro and macronutrients, sewage sludge can serve as cheap and efficient feedstock which, when combined with additional carbon source, can deliver high SCO yields while maintaining low production costs.

3.2. Carbon Compounds

Butyric acid, acetic acid, glycerol and other organic compounds are generated in various industries as products, by-products or residues. These compounds can be directly used as carbon and amino acid sources for SCO production, while at the same time enabling the treating of industrial effluents.

3.2.1. Glycerol

In the last 15 years the production volumes of biodiesel have increased significantly, due to the compatibility of biodiesel and its production technology with the already existing fuel industry infrastructure, the relatively simple production process, the possibility of using various raw materials in the production process and subsidies to producers [58]. Global biodiesel production in 2014 reached 26.5 million tonnes a year, which is more than a 4-fold increase, when compared with the production volumes in 2006 [58], [59]. Even though this increase is a good example of greenhouse gas emission level reduction and an example of renewable energy sector development [60], biodiesel production creates 100 kg of crude glycerol per each tone of biodiesel [2]. It means that each year more than 2 million tons of glycerol is being produced as a biodiesel production by-product [61].

Crude glycerol, which is being created during the biodiesel production process, contains such impurities as alcohols, heavy metals, water and various salts, which is the reason why it is necessary to treat industrial glycerol prior to its utilization in other sectors [2], [61]. Due to the impurities, transportation costs and low market prices, market for untreated glycerol is limited. As a result, biodiesel producers often sell glycerol as a fuel, which is a solution associated with low profit margins. New and innovative technological solutions and an introduction of more effective glycerol utilization solutions could help in further development of the whole biodiesel industry. In SCO production both treated and untreated glycerol can be used [62], resulting in overall reduction of production costs, since the untreated glycerol has a very low market price (2–5 EUR per kilogram) [63] and a relatively small amount of glycerol must be added into the medium (20–100 g/L). Glycerol utilization in SCO production offers an opportunity to produce a product with high added value and enables more profitable utilization of glycerol. Utilization of glycerol in SCO production is widely described in numerous publications [28]–[38] with extremely high SCO yield reported when using *Schizochytrium sp.* (Table 1).

3.2.2. Sodium Gluconate

Sodium gluconate is a sodium salt of gluconic acid, which is very soluble in water and is therefore found in industrial wastewaters where it is produced and used. Sodium gluconate is widely used in the textile industry, dyeing, printing, metal surface treatment as well as a cleaning agent and as a chelating agent. Gouda et al. reported that sodium gluconate can be used as a carbon source for oleaginous bacteria *Gordonia sp.* and *Rhodococcus opacus* cultivation [39].

3.2.3. Butanol Wastewaters

In order to replace the production of butanol from petroleum, it can be produced using an acetone-butanol-ethanol fermentation process in which anaerobic bacteria ferment carbohydrates and synthesize acetone, butanol and ethanol. This process is environmentally friendly, uses renewable resources such as starch or glucose and eliminates the need for fossil resources. After fermentation, more than 99 % of the produced acetone, butanol and ethanol are recovered from the fermented culture medium, but the used medium (butanol wastewater) is characterized by high chemical oxygen demand (COD) values (23560 ppm) due to residual carbohydrates and organic acids such as acetic and butyric acid [40]. In order to treat butanol wastewater and reduce the negative environmental impact that these residues would have if they entered the environment previously untreated, they can be used as a medium base for cultivating oleaginous microorganisms. Without addition of additional nutrients Peng et al. [40] cultivated *Trihosporon dermatis* in butanol wastewater and reported relatively low SCO yield, while significantly reducing COD levels in the wastewater. In the future, combination of butanol wastewater with other carbon-rich waste products could ensure significant increase in SCO yields.

3.3. Sources for Photosynthetic Microorganisms

3.3.1. CO_2 as Carbon Source

Today it is well known as CO_2 becomes more potent in atmosphere, overall temperatures keep rising. Due to CO_2 effects on climate change, there are more and more attempts to remove CO_2 from the atmosphere. Hence carbon capture and storage (CCS) technologies have been developed in recent years [64], technologies like CO_2 capture by ionic solvents and its storage deep in the ground, so that it cannot re-escape into the atmosphere [65]. On the downside, these technologies require a lot of energy. Alternatively, autotrophic organisms, such as plants and microorganisms can be used to assimilate CO_2 into biomass by photosynthesis [66]. As mentioned above, microorganisms are more effective for CO_2 capture. In line with this review, we analysed the potential of CO_2 usage as a carbon source for SCO production. Industries such as steel and oil refining, coal and natural gas, as well as fertiliser production are the main producers of CO_2 [65], [67].

There have been attempts to develop an economically feasible system for CO_2 assimilation by using algae simultaneously for effluent wastewater treatment from steel production plants. [68] Due to the low C/N ratio of wastewater [69], autotrophic microorganism cultivation would be suitable in such case. As mentioned above, one of the main carbon dioxide producing industries is the fertiliser production industry [70]. According to the Centre for

European Policy Studies report from 2014, more than 130 million tonnes of ammonium were produced globally in 2012, with the main producers being China with 44 million tonnes, India 12 million tonnes and Russia with 10 million tonnes [71]. The Intergovernmental Panel on Climate Change (IPCC) in 2006 published Guidelines for National Greenhouse Gas Inventories, in these guidelines IPCC states that 2.104 tonnes of CO_2 are produced from every ton of ammonium produced [72]. Although IPCC calculations are derived from European average values for specific energy consumption (mix of modern and older plants), assuming this emission factor is applicable on the global scale, we can calculate that 274 million tonnes of CO_2 are produced every year from ammonia production alone. This is an untapped resource for SCO production autotrophically.

In addition to light, source of nitrogen and carbon dioxide, algae need phosphorus and potassium. Recommendations of N/P/K proportions vary from equal amounts of nitrogen, phosphorous and potassium for *C. vulgaris* cultivation [73] to a N/P/K ratio of 6/1/1 for *Dunaliella tertiolecta* cultivation [74]. Standard mediums, like Soil extract [75] and Bold's Basal Medium [76] for laboratory cultivation give ratios of 1/1/2, leaving phosphorus as limiting. The amount of potential biomass yield from certain amounts of CO_2 are described by two stoichiometric equations, depending on nitrogen source used [77], [78]:

$$0.81\ CO_2 + 0.73\ H_2O + 0.12\ NH_3 + \text{minerals} \xrightarrow{hv} 0.81\ \text{C-mole} + O_2, \tag{1}$$

$$0.71\ CO_2 + 0.59\ H_2O + 0.101\ NO_3^- + \text{minerals} \xrightarrow{hv} 0.71\ \text{C-mole} + O_2. \tag{2}$$

These equations can give a rough estimate of the required amount of nitrogen, other essential elements are excluded from these equations due to negligible amounts of these elements as seen in the elemental composition of green algae *C. vulgaris* $C_{60}H_9O_{21}N_{6.5}P_{0.8}S_{0.08}$ [79], as according C-mole of *C. vulgaris* is $CH_{0.15}O_{0.35}N_{1.08}P_{0.01}S_0$. This leads to nitrogen and phosphorus being the main nutrients causing algae blooms and eutrophication by wastewaters flushing into natural bodies of water. So it makes perfect sense that microscopic algae have already been used for tertiary wastewater treatment [80]. In this case, inorganic nitrogen and phosphates are used as valuable nutrients for algal growth. Tertiary treatment of wastewater is often skipped due to high expenses [81], though if oleaginous algae as *C. vulgaris* is chosen for this treatment, financial gains could be potentially made after biomass collection and oil extraction. As *C. vulgaris* in autotrophic growth conditions can yield 38 % lipids of its dry cell weight [82]. In addition, similar to algal single cell protein production, costs of SCO production from algae can be lower due to the fact that at least part of the required nutrients are provided by waste [83] – wastewater and CO_2 emissions.

According to a 2016 study looking into Barcelona's (Spain) wastewater treatment plant (WWTP), after secondary treatment a considerable amount of potassium, phosphates, nitrates, and ammonium are present in the effluent – 36, 14, 51 and 30 mgL^{-1} accordingly [84]. This gives a rough N/P/K ratio of 9/1/9. As mentioned above, N/P/K ratios vary considerably, in Collet et al. study *C. vulgaris* was cultivated [85] in open raceways with N/P/K ratios being closer to 8/2/1, N/P ratio in this case is close to abovementioned effluent from secondary wastewater treatment plant in Barcelona [84]. This would leave excess potassium in the effluent from tertiary treatment. This would leave SCO producers with choice – to supplement secondary wastewater effluent with additional nitrogen and phosphorus source or let the extra potassium go to waste and avoid extra costs. According to Yakushev et al. research, 229 hm^3 of wastewater is treated in the same Barcelona WWTP in a single year, roughly 0.627 $hm^3 \cdot day^{-1}$ [86]. By using two abovementioned Eq. (1) and Eq. (2) for biomass production from NH_3 and NO_3^- accordingly, required CO_2 can be calculated. As a result, 504.8 g L^{-1} of CO_2 is needed for complete removal of NO_3^- from medium, and an additional 494.2 g L^{-1} of CO_2 for complete NH_3 removal. Assuming there is no deficit of other nutrients, 229 kilo tonnes of CO_2 could be assimilated in a course of a year from the abovementioned WWTP, in these conditions 445 g of *C. vulgaris* biomass could be produced from every 1 kg of carbon dioxide.

4. CONCLUSIONS

In the scope of this review only a few industrial by-products were considered for SCO production. As shown in this review, every waste or by-product could be used for higher value-added product production, as well as for reduction of an industry's burden on the environment as in case with wastewaters, carbon dioxide and butanol wastewaters.

When considering waste and by-product usage for SCO production, a couple of things should be taken into account:

– Oil quality and purity requirements;
– Desired fatty acid profile and the respective microorganisms and their requirements for growth and oil production conditions;
– Additional expenses for substrate pre-treatment and transportation;
– Concentration of potential inhibitors.

There is no defined answer to what would be the best substrate for SCO production, as all abovementioned criteria should be considered. For example, carbon dioxide would require the least pre-treatment, but for economic reasons it should be coupled with effluents from secondary wastewater treatment, and only photosynthetic microorganisms could be cultivated this way – this leads to demand of vast land areas, as with today's technologies net energy ratio is greater than one only in open pond cultivation systems [87]. When considering PMS hydrolysate, not only additional costs of pre-fermentations should be taken into account, but also inhibitor presence [88] and effects on chosen microorganisms. As mentioned in previous studies [1], [7], [13], [17] each waste material must be evaluated not only in regard to its economic feasibility, but should be compared to already existing or potentially emerging competing sectors.

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