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ScienceDirect

Energy Procedia 147 (2018) 409–418

Energy Procedia

www.elsevier.com/locate/procedia

International Scientific Conference "Environmental and Climate Technologies", CONECT 2018

Single cell protein production from waste biomass: comparison of various industrial by-products

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Abstract

Industrial waste accounts for a considerable amount of manmade waste streams, of which it is the most distinctly harmful to local environments if released untreated. Microbiological treatment of biodegradable waste materials ensures neutralization of harmful substances and allows for the reduction of environmental pollution. Additionally, conversion of these wastes into value-added products enables particular recycling efforts to become more economically viable. Single-cell protein is one such value-added product that can be derived from various waste materials via microbial fermentation. In this review various biodegradable industrial by-products as substrates for production of SCP are categorized and compared.

Keywords: single cell protein; waste biomass; industrial by-products; resource availability; aquaculture; fish feed; fish meal

1. Introduction

The recycling of waste helps to reduce negative environmental impact and reduce the overall costs associated with waste management. However, waste recycling is no longer considered to be a sufficient waste management practice and, nowadays, due to environmental, governmental and economic pressures, waste treatment needs to be combined with the production of value added products. Currently, a large proportion of biodegradable waste is simply burned

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10.1016/j.egypro.2018.07.111

[1] or processed into products with a relatively low added value, such as biogas [2], bioenergy and biofuels [3, 4]. However, some technological developments have enabled the production of high added value products from these waste materials [5–8]. One of these high added value products is single-cell protein (SCP).

SCP is microorganism biomass or protein extract derived from microscopic algae, yeast, fungi or bacterial cultures, which can be used in animal and human nutrition [9]. SCP is a good alternative for substituting agricultural proteins, since SCP production is more environmentally friendly [10], consumes less water [11], requires smaller land areas and its effect on climate change is much less pronounced [12] than it is in the case of agriculturally derived proteins. Another advantage of SCP is that it is possible to use a wide variety of biodegradable industrial wastes for the cultivation of SCP producing microorganisms. The use of waste products in the production of SCP can reduce the production costs and waste treatment reduces the negative environmental impact of these residues.

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.

This review is a follow-up to Spalvins et al. [14] on agricultural waste products suitable for SCP production. In other reviews [9, 15, 16], which summarize the reported findings on the waste products suitable for the cultivation of SCP producing microorganisms, information mainly focuses on the used microorganisms and not so much on the properties of the waste products themselves. However, nowadays, for both research and industrial applications, access to various strains of microorganisms is relatively simple, but availability of waste products is very specific for every local economy. Consequently, in order to further facilitate the identification of the most suitable waste products, this review seeks to categorize and describe industrial waste products suitable for the production of SCP [14].

2. Waste types

Spalvins et al. [14] reviewed the most suitable agricultural wastes for SCP production. According to the definition of industrial wastes, a number of agricultural wastes can also be categorized as industrial ones because they come from industrial processing plants, however, in the context of these reviews, they were categorized as agricultural, because the main resource used in the generation of waste was originally grown on agricultural lands. This paper will review industrial waste products that are not directly related to agriculture or food production.

By reviewing available literature on the use of industrial waste products suitable for production of SCP, it was possible to categorize industrial wastes in three groups of by-products: polymer-rich sources (industrial waste); carbon compounds; sources for photosynthetic microorganisms (Fig. 1).

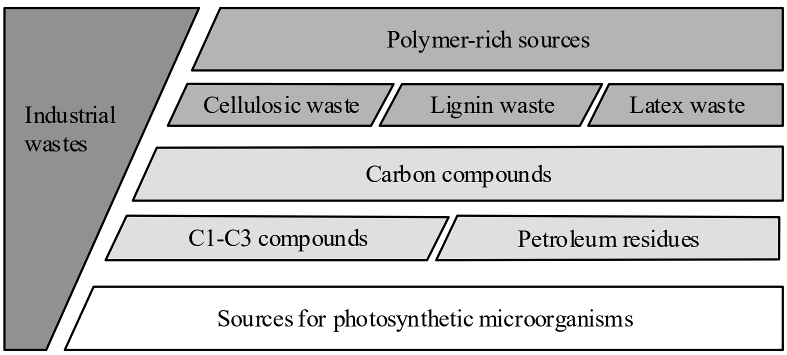

Fig. 1. Categorization of industrial wastes applicable for cultivation of SCP producing microorganisms.

3. Comparison of waste resources

Industrial waste resources applicable for SCP production reviewed in this paper are summarized in Table 1.

Table 1. Industrial wastes applicable for SCP production. Recent reports of protein content (% of biomass after fermentation).

Polymer-rich sources	Microorganisms	Protein content, %	Ref.
Waste paper	*Scytalidium acidophilum*	47	[18]
Sulfite waste liquor	*Paecilomyces variotii*	55	[35]
	Fusarium venenatum	55	
	Candida utilis	-	[20]
Lignin residues	*Chrysonilia sitophila*	39.2	[40]
Latex rubber sheet wastewater	*Rhodopseudomonas palustris*	65	[47]
Carbon compounds			
Methane	*Mehtylomonas sp.*	69.3	[56]
	Bacteria isolated from soil	71	[53]
Methanol	*Methylophilus methylotrophus*	-	[86]
	Pichia pastoris	35	[87]
Acetic acid Formic acid	Bacteria isolated from soil	71	[53]
Waste gases	*Methylococcus capsulatus*	-	[69]
	Rhodobacter capsulatus	-	[70]
Glycerol	*Acinetobacter baylyi*	-	[80]
	Aurantiochytrium limancinum	-	[81]
	Schizochytrium limacinum	-	[82]
Gas oil n-paraffins	BP yeast	67–69 63	[83]
Sources for photosynthetic microorganisms			
Effluents of biogas plants	*Rhodopseudomonas capsulate*	69.4	[88]
	Rhodopseudomonas spp.	49–68	
Saline sewage effluents	*Chlorella salina*	51	[89]
Wastewater effluents	*Micractinium*, *Scenedesmus* *Oocystis*, *Franceia* *Euglena*	40–60	[90]

3.1. Polymer-rich sources (industrial waste)

Cellulose, lignin, hemicellulose, latex and other polymers as waste are accumulated from wood and cotton processing, paper and fuel production, latex and other industrial processes [17]. Polymers, especially lignocellulosic waste, are the most widely available industrial waste. However, polysaccharides and other complex compounds require a thorough mechanical, chemical or enzymatic pre-treatment (or combination of treatments) before the SCP producing microorganisms can ferment them. Consequently, the use of polysaccharides increases the cost of SCP production.

3.1.1. Cellulosic waste

Cellulose is the main component of agriculture, wood and municipal waste [18, 19]. Cellulose-rich waste is also generated by wood processing and paper industries [20]. The most widely known pre-treatment methods for cellulose-rich wastes are microbial cellulolysis using cellulolytic microorganisms [21, 22], mechanical thermolysis of cellulose using steam [18, 23], chemical dissolution using acids and alkali [24, 25] or biochemical digestion using enzymes [18, 26]. After applying one or multiple pre-treatments, waste can be further fermented by SCP producing microorganisms.

3.1.1.1. Waste paper

In 2014, 390 million tons of paper and cardboard were produced globally [27]. Currently, in developed regions such as Europe and North America, the average paper recycling volume varies around 60–70 % of the total paper output [29, 30]. However, in some developed and developing countries, paper recycling rates are much lower [31, 32], and paper production is projected to reach 490 million tons a year in 2020 [28]. Consequently, the amount of waste paper available is very high and in the future it is predicted that it will continue to grow.

The main component of paper waste is cellulose. Hydrolyzed paper waste contains about 60–70 % sugar, of which about 70 % is glucose, 20 % xylose, 3 % mannose, 3 % arabinose, 1 % galactose, and other 30–40 % consists of 20 % lignin, 60 % clay mineral kaolinite, and other residues [18]. Usually SCP production from waste paper is encumbered by contamination of unwanted microbial material. Thus maintenance of contamination increases the total cost of production [28]. In order to avoid these costs, a good alternative is the use of extremophilic microorganisms in the production of SCP [18], therefore selective growth conditions could be maintained without the risk of contamination.

3.1.1.2. Sulfite waste liquor

Spent sulphite liquor (SSL) is a by-product of the sulfite process, which produces wood pulp from wood chips. SSL can be used effectively in the cultivation of microorganisms, and it is possible to obtain ethanol [33], vanillin [34], SCP [20, 35] and other products from SSL [36].

Since the 1930s, the sulfite process has been slowly replaced by the Kraft process, which allows to recycle almost all chemicals used in pulping [33], sulfite process is used by less than 10 % of the chemical pulps today [37, 38]. If sulfite pulp mills are locally available, the use of SSL as substrate for SCP production can be considered as promising waste material since SSL is cheap and locally abundant. SSL fermentation also reduces biological oxygen demand (BOD), therefore SSL fermentation allows not only to produce high amounts of value added SCP [35, 39], but also reduce environmental harm [20].

About 10 % of SSL are dissolved solids that contain lignosulphonates and by-products of hemicellulose hydrolysis [20]. Hemicellulose residues contain about 30 g/L of hexoses and pentoses, which microorganisms can easily access [20, 39].

3.1.2. Lignin waste

Lignin, cellulose and hemicellulose are the main components of wood. In plants cellulose fibers are surrounded by lignin, pectin and hemicellulose, which increase the mechanical strength of the cellulose and protect it against microorganisms [40, 41]. Lignin is one of the main waste products in the paper industry, which after removal from cellulose is burnt as a fuel, which is a very low added value solution. Consequently, there is a potential to find economically more profitable applications for lignin. Lignin residues can be used in microbial fermentation and there are a number of microorganisms that are able to degrade lignin and use it in fermentation [40]. However, compared with other waste products, the digestion of lignin residues is slow and accumulated amounts of SCP are relatively small.

Lignin degradation makes polysaccharides in fibers more accessible to microbiological fermentation and, in general, lignin digestion is a topical issue in the paper industry, in processing of agricultural waste, in production of various chemicals and in neutralization of contaminants [40, 42, 43].

3.1.3. Latex waste

Latex is a complex mixture of substances consisting of proteins, saccharides, alkaloids, oils, resins, gums and tannins [44]. Nowadays latex is mainly derived from plants (*Hevea brasiliensis*, *Landolphia genus*, etc.) and is used to produce latex rubber [45]. Latex rubber is a polyisoprene polymer, which is an elastomer and a thermoplastic material, which once vulcanized becomes thermoset [46].

Rubber sheet production is very common in regions where latex rich plants are cultivated [47, 48]. Latex rubber production generates wastewater that is rich in ammonia, formic acid, sodium metabisulfite and sodium sulfite [47, 49, 50]. As these wastewaters are hazardous to the environment; therefore, it is necessary to treat them before discharging them into local water bodies. Currently lagoons and oxidation ponds are used for latex rubber wastewater treatment, but these systems are ineffective, because they do not completely oxidize compounds present in the wastewater. Use of these basic treatment systems causes an irritating odor and releases into the atmosphere such greenhouse gases as methane and carbon dioxide [51]. Consequently, specialized fermentation of this wastewater can be a good alternative to the technologies used currently.

It has been reported that the use of latex rubber sheet wastewater in the production of SCP can yield high protein concentrations in the microbial biomass and also considerably reduce chemical oxygen demand (COD) values [47].

3.2. Carbon compounds

3.2.1. C1-C3 compounds

Methane, methanol, acetic acid and formic acid are commonly found in the environment. These compounds are mainly produced by the decomposition of organic compounds, as well as by biochemical processes found in plants and animals [52]. Under industrial conditions, these substances accumulate during storage or processing of various resources and waste products, hence the effective use or safe treatment and disposal of these compounds is vital.

3.2.1.1. Methane

Methane, as a cheap and widely available carbon source for the production of SCP, has been studied for a long time [53–56]. Although methane is highly flammable, poorly soluble and naturally occurring with different impurities, its benefits in the production of SCP are its selectivity, low toxicity and volatility [56, 57]. Natural gas, due to its high methane content (85–90 %) is considered to be one of the most suitable sources of methane for the production of SCP [56]. So far, the use of natural gas for cultivating experiments has resulted in the production of microorganism biomass with very high concentrations of SCP [53, 56].

Data provided by the U.S. Environmental Protection Agency [58] shows that the natural gas industry, due to venting, flaring and leaks within the industry's operations, waste more than 16 billion cubic meters of natural gas annually in United States alone [59]. It is a huge amount of wasted gas, which not only creates huge economic losses, but also releases huge amounts of methane, which is a significant greenhouse gas with a much higher global warming potential than carbon dioxide [60]. Although gas leakage within the natural gas industry is outside of the scope of this review, these leakage estimates show that waste gas is available in enormous amounts and can be used to produce SCP.

3.2.1.2. Acetic acid

Annually around 6.5 million tonnes of acetic acid are consumed [61]. Acetic acid is widely used in the production of various polymers, glues, fibers and fabrics, as well as in food production as an acidity regulator and in household cleaning as a descaling agent. From these industries large amounts of acetic acid enter wastewaters and cause environmental problems to local ecosystems [62]. Currently extensive research is devoted to efficient and inexpensive recovery of acetic acid from wastewaters; however, more widely used methods are chemical or physical-chemical bonding or distillation [62–65]. Although the use of acetic acid in the cultivation of microorganisms is scarcely described, acetic acid as a carbon source has been reported to show rapid growth rates of microbial biomass [53]. In the future it is required to investigate more closely the use of acetic acid-rich wastewaters in cultivation of SCP producing microorganisms.

3.2.1.3. Formic acid

Large quantities of formic acid are generated as a by-product from the production of other chemicals [61]. Consequently, a significant amount of formic acid is introduced into the industrial wastewaters which need to be treated. Formic acid can be bound to sewage in the activated sludge process [62, 66, 67] and it can be used by microorganisms as a carbon source [53].

3.2.1.4. Waste gases

Waste gases are an innovative source of nutrients. Gas-fermenting microorganisms are able to use carbon monoxide (CO), carbon dioxide (CO_2) and methane (CH_4). These gases are generated from steel and oil refining, coal, natural/shale gas and syngas industries. The use of waste gases allows for a significant reduction in emissions of greenhouse gases and production of value-added products such as chemicals (acetic acid) [68, 69], fuel (botryococcene) [70] and nutrients (SCP) [69].

Waste gases can also be used for cultivation of photosynthetic microorganisms (see subchapter 3.3).

3.2.1.5. Glycerol

Thanks to compatibility with existing fuel infrastructure, a relatively simple production process, and the ability to utilize a variety of feedstocks and substantial subsidies from local governments, biodiesel production volumes have been rapidly increasing for the last 15 years [71]. In 2014 global biodiesel production increased to 26.5 million tons, which is more than 4-fold increase when compared to production levels of 2006 [71, 72]. Although this increase in production volumes is welcomed in regard to reduction of greenhouse gas emissions [73], biodiesel production generates 100 kg of crude glycerol from every tonne of biodiesel [1]. This means that more than 2 million tons of glycerol are generated annually from biodiesel production alone.

Glycerol can be used in production of various foods and beverages, chemicals, pharmaceuticals, cosmetics etc. However, it is predicted, that production of biodiesel will continue to increase rapidly in the future, but already at the moment, available volumes of glycerol are enough for industries, where glycerol is used [1]. Therefore, a problem is developing, where conventional sectors which used most of the available glycerol will not be able to accommodate for excess glycerol in the near future.

Crude glycerol, which is generated during production of biodiesel, contains impurities such as alcohol, heavy metals, water and various salts, so it is necessary to purify industrial glycerol before it can be used in other industries [1, 74, 75]. Due to the impurities, transportation costs and low market prices, biodiesel producers have a limited ability to sell generated crude glycerol [1]. Therefore, manufacturers often sell it as a fuel or as a feed additive, which is a low added value solution. New and innovative technological solutions and more efficient glycerol commercialization can contribute to the development of the entire biodiesel industry. The use of glycerol in the production of SCP would provide the opportunity to produce a high value added product and allow the biodiesel industry to get additional revenue by using glycerol more economically efficiently. The use of glycerol for the cultivation of microorganisms, including SCP production, is covered in multiple publications by different authors [76–82].

3.2.2. Petroleum residues

Hydrocarbons, which are generated from petroleum refineries, can serve as an alternative to more conventional carbohydrates. Unlike carbohydrates, hydrocarbons have no oxygen and are practically insoluble in water [83]. The use of petroleum residues in SCP production provides an opportunity to reduce the environmental impact caused by the oil industry, although, in the fermentation process only a small proportion of hydrocarbons is used as feedstock [84]. SCP producing microorganisms can use fuel oil and other n-parafins, which are distillates or residues from petroleum distillation [83–85].

3.2.3. Other carbon compounds

It has been reported that other compounds such as methanol, hexane, heptane, heptanol, nonanol, necanol, propionic acid, caprylic acid and capric acid can be used in production of SCP [53, 84, 86].

3.3. Sources for photosynthetic microorganisms

Algae, zooplankton and bacteria are widely used in the treatment of wastewaters and other liquid wastes where microorganisms are cultivated in special ponds or lagoons [87–89]. These microorganisms bind and use the inorganic nitrogen and phosphorus in the residue, in addition, sewage-grown algae, bacteria and zooplankton are a good source of protein that can be used in animal feed. Depending on the selected strain, microorganisms can grow on either freshwater or high salinities sewage effluents [89]. An efficient approach on sewage effluent treatment is using cooperative cultures of organic material oxidizing bacteria, which releases CO_2 and algae which can assimilate generated CO_2 and during daytime release O_2 through photosynthesis.

It has been reported that high SCP concentrations have been obtained from such wastewaters as saline and freshwater domestic sewage effluents and effluents from biogas plants [88–90].

4. Conclusions

In this review, most of the industrial wastes that can be used in the production of SCP have been categorized and discussed more closely. Each industrial waste group has its own advantages and disadvantages if used as substrate for SCP production. Use of polymer-rich sources is problematic mostly due to extensive pre-treatments these wastes require before efficient SCP fermentation can take place. Carbon compounds, especially waste gases and glycerol, have the highest potential in becoming widely used carbon sources for various types of microbial fermentations, including SCP production, but further advancements in these technologies are required for these sources to become more widely accepted. Basic infrastructure for using various wastewaters for SCP production already exists; however, reasonable concerns over heavy metal and other admixtures in biomass and inefficient waste and biomass separation solutions are holding back the use of wastes applicable for photosynthetic microorganisms.

The key considerations for choosing the most suitable waste product for SCP production remains the same as concluded in previous review [14] with few additions. Key considerations are:

- 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 substrate;
- SCP 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 methods;
- SCP extraction (protein extraction from biomass and removal of impurities) methods.

In the future, it is also necessary to thoroughly review and compare the different agro-industrial wastes in regard to their use as a substrate for single-cell oil (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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