EXOPOLYSACCHARIDES SYNTHESIS ON INDUSTRIAL WASTE

T. P. PIROG, M. O. IVAKHNIUK, A. A. VORONENKO

National University of Food Technologies, Kyiv, Ukraine

E-mail: tapirog@nuft.edu.ua

Data from the literature and our own studies on the synthesis of microbial exopolysaccharides on various industrial waste (food industry, agricultural sector, biodiesel production, etc.) are reviewed here. Utilization of industrial waste to obtain exopolysaccharides will solve not only the problem of secondary raw materials accumulation, but also will reduce the costs of the biosynthesis of practically valuable metabolites. In addition, some kinds of waste have a number of advantages compared to traditional carbohydrate substrates: aside from environmental health benefits, there are technological ones, like the presence of growth factors. There is also no need to use anti-foam substances and substrate sterilization in the latter case.

Despite a longer than forty years history of researches, microbial exopolysaccharides (EPS) — high-molecular exogenous microbes' metabolism products of carbohydrate nature — remain objects of in-depth studies. Due to their abilities to change rheological features of aqueous systems, emulsifying, suspending, gelling, they are widely implemented in petroleum, mining, food, perfume, chemical, textile industries and agriculture [1−4]. Aside from that, in recent years significantly increased the number of studies on their use in the fields of pharmacy and medicine [5−7]. Microbial EPS have a number of advantages over synthetical and plant-derived polysaccharides, as they are non-toxic and biodegradable, resistant to mechanical and oxidizing destruction, temperature and low pH values [8, 9].

Demand for microbial polysaccharides on the world market is high, which is supported by the year-to-year growth in the production of xanthan gum, the first microbial EPS (produced by *Xanthomonas campestris*, isolated in the late 1960s) [10]. Plenty of research labs in leading countries of the world look for novel EPS with unique properties, in particular, which are synthesized by extremophilic microorganisms [11−13].

However, despite the longstanding history of microbial EPS researches, so far only carbohydrate substrates have been mostly used for their biosynthesis. In the industrial conditions of EPS production, usually products derived from sugar-beets: molasses, sugar syrup, sucrose, or from corn: starch, hydrolyzed starch, glucose syrup, glucose, maltose are used as the substrates [8, 14, 15].

Studies conducted as early as 1970s−1980s, demonstrated that the substrate variety for microbiological production of EPS can be augmented by including non-food substrates (methane, methanol, ethanol, ethylene glycol, carbohydrates) [8]. Despite that, there are limited findings about EPS synthesis on non-carbohydrate substrates.

One of the current global environmental problems is the need for ways of utilization or recycling industrial waste. Notably, not only waste which content toxic substances are dangerous (for example, phenol and its derivatives), but also others entering the environment in uncontrolled quantities, such as oil-containing ones (waste of oil-fat production, fried oil from catering, etc.)

Biotechnology lets us to utilize the waste and obtain valuable biologically active substances or biomass.

In our previous reviews [16, 17] we summed up the data on the synthesis of microbial surfactants and other products (alcohols, organic acids, polyhydroxyalkanoates) on industrial waste. Note, that to this day, the literature data about synthesis of microbial EPS on such substrates are extremely limited.

The aim of the review is to survey the results obtained in recent years considering the synthesis of exopolysaccharides of microbial origin on industrial waste.

Waste of sugar industry

The waste of sugar industry (molasses and sugar syrup), unlike other kinds of industrial waste, have already been widely implemented in the synthesis of microbial EPS, since they are composed mostly of carbohydrates. The latest literature contains reports of using molasses and sugar syrup as substrates in the production of various microbial polysaccharides. The advantages of such waste are the high fractions of sucrose and other carbohydrates, low cost, availability and durability in storage. Since molasses contain organic nitrogen, high content of the substrate in the cultivation medium lowers the amount of additional nitrogen source to include.

Moosavi and Karbassi [18] described a way to use molasses as the only source of carbon and energy for xanthan synthesis by a wild strain of *Xanthomonas campestris* pv. *campestris*. If the producer was cultivated in a medium with 5% of molasses, the amount of synthesized EPS was 19.8 g/l.

Abdel-Aziz et al. found that the fungus *Mucor rouxii* NRRL 1894 can synthesize EPS (7.2 g/l) in a medium with 30 g/l of crude molasses. The authors state that the high yield of the polysaccharide resulted from the presence of both the available carbohydrates and ions of Fe^{2+} and Zn^{2+} in molasses composition, which can be stress factors leading to increased synthesis of EPS [19].

In data [20] was shown that strain *Xanthomonas campestris* ATCC 1395 was able to synthesize xanthan using molasses as substrate. Maximal content of EPS was 53 g/l on a medium with 175 g/l molasses.

Küçükaşik et al. [21] used beet molasses or molasses-starch mixture, instead of sucrose, as carbon source for *Halomonas smyrniensis* AAD6 to produce levan. In a medium with 50 g/l of sucrose the producer synthesized 1.84 g/l of levan, while on sugar-beet molasses (30 g/l) the EPS content reached to 12.4 g/l.

Sugar-beet molasses was used as sucrose alternative substrate for *Bacillus subtilis* WCS36 strain. The amount of synthesized polysaccharide was 4.86 g/l when the producer grew in a medium with 2% molasses, which is 1.5 times more than when sucrose was used [22].

Cane molasses — waste of sugar cane industry, can be also used to synthesize polysaccharides. Under *B. subtilis* 168 cultivation in a medium with 2.36% of the substrate, 4,92 g/l of EPS were produced [23].

There's a report of pullulan synthesis process by a fungus *Aureobasidium pullulans* MTCC 1991 grown in medium with sucrose (40 g/l) or molasses (50 g/l) [24]. The EPS content in both media was equal — 27.3–27.5 g/l.

To produce levan, strain *Zymomonas mobilis* ATCC 31821 was grown on molasses and sugar syrup (250 g/l). When molasses was used, the bacteria produced 2.53 g/l of levan, while on a medium with sugar syrup the EPS content was 15.5 g/l [25].

Sclerotium rolfsii MTCC 2156 produced 23.87 g/l of scleroglucan during 72 hr of cultivation on a medium with 120 g/l of sugar syrup. Under the same content of beet molasses, the polysaccharide yield was somewhat lower (19.21 g/l) [25].

When grown in a medium with 2% molasses, *Sphingomonas paucimobilis* ATCC 31461produced up to 13.82 g/l of gellan [25].

It has been reported [26] that if *Bacillus subtilis* was cultivated on 2% molasses, EPS content was maximal (5.56 g/l).

The efficiency of molasses transforming into EPS is comparable to the values for traditional carbohydrate substrates (sucrose, glucose). Thus, EPS yield (the ratio of the synthesized EPS concentration to the concentration of substrate in g/l) is on average 20–40% if the producer is grown either on molasses or on carbohydrates. Also, molasses using as EPS biosynthesis substrate is limited by the presence of possible microbial growth inhibitors (betaine, metal cations), as well as the necessity to pre-hydrolyze it for the producers who lack invertase.

Whey

Whey is the main by-product of dairy industry, contains high levels (70%) of lactose. Aside of milk protein (another important constituent), whey also has nutrients and growth factors, which can serve to activate

the synthesis of the final product [27−29]. The necessity to utilize it is an urgent problem for the dairy industry, since the daily world production of whey is, on average, 500 m^3 [27]. It is often poured into rivers and lakes, which lowers the oxygen levels and leads to the death of many water organisms. There is a threat of polluting ground waters by this waste [30, 31]. Whey use as a growth substrate in microbial synthesis is bounded by the fact that only a few microorganisms (lactic acid bacteria, enterobacteria, yeasts of the genus *Kluyveromyces*) synthesize the enzyme necessary to hydrolyze lactose (β-galactosidase).

As early as 1990s, a gene-engineered strain of *X. campestris* was created, able to assimilate whey lactose as carbon source [30]. For this purpose, genes *lacZY* of *Escherichia coli* that code β-galactosidase and lactosopermease were carried in a plasmid with a wide range of hosts so that they would be controlled by transcriptional control of a promoter of one of the *X. campestris* bacteriophages. The construct was entered into *E. coli*, and then carried from *E. coli* to *X. campestris* by triple crossing. The transformants which had the plasmid synthesized β-galactosidase and lactosopermease, using lactose as the only carbon source and synthesized xanthan on a medium with glucose, lactose and whey [30]. However, the concentration of xanthan synthesized by the gene-engineered strain on whey wasn't high — only 4.2 g/l.

Twenty years later, in 2011, there was a report about 12 g/l of xanthan synthesize by strain of *X. campestris* Tn-UV-2 on this substrate [31]. To obtain *X. campestris* Tn-UV-2, the genes for β-galactosidase synthesis were carried from *E. coli* cells into *X. campestris* using plasmids pSUP5011. Strain TC 49, which synthesized the highest content of the final product, was UV-irradiated. The obtained gene-modified mutant Tn-UV-2 produced almost 12 g/l of xanthan under its cultivation on whey [31].

There is a study [27] on cultivating xanthan producer *X. campestris* ATCC 13951 in a medium with three kinds of whey: deproteinized, partially hydrolyzed, hydrolyzed and deproteinized. Strain ATCC 13951 in the medium with hydrolyzed whey (43 g/l by lactose) synthesized 28 g/L EPS, while on the two other kinds of whey no EPS synthesis was observed [27].

Gilani et al. [28] established that the maximal content of xanthan, produced under *Xanthomonas campestris* PTCC 1473 cultivation on whey (30 g/l by lactose) was 16.5 g/l. To deproteinize whey it was cooled to 0−5 °C, then CaCl$_2$ was added and the whey was neutralized, and lastly heated to 50 °C, and the precipitate removed by centrifugation [28].

Other authors [20] determined the optimal whey concentration for xanthan synthesis by *X. campestris* XLM 1521. They found that the maximum amount of EPS (12 g/l) was obtained under strain XLM 1521 cultivation in a medium with 50% whey (v/v).

Gellan producer *S. paucimobilis* ATCC 31461 was cultivated in a medium with glucose (5−30 g/l), lactose (5−30 g/l) or whey (52 and 0.5 g/l by lactose and milk acid, respectively). It was established that when whey was used, strain ATCC 3146 produced only 7 g/l of EPS, but the synthesized gellan was less degradable by bacterial enzymes than obtained on lactose and glucose [29].

Another study [32] reports the ability of two strains of medicinal fungi *Ganoderma applanatum* 1572 and *G. lucidum* 1621 to synthesize EPS on whey from Public joint-stock company Yagotynsky butter plant (Yahotyn, Kyiv region). On the 11th day of cultivation, the concentration of EPS synthesized by *G. applanatum* 1572 was 9.1, and that of *G. lucidum* 1621 was 10.0 g/l. The whey used for EPS synthesis contained (%, w/w): lactose — 60; protein — 10; lipids — 2; lactic acid — 7.85; vitamins — 0.15; ash — 7 [32].

There is a strain of *Zunongwangia profunda* SM-A87 which synthesized 12.1 g/l of EPS on a whey-containing medium (60.9%, v/v) [33].

Lactic acid bacteria *Streptococcus thermophilus* are used in the production of cheeses and yoghurts. Strain *S.thermophilus* BN1 produced 548, 325 and 375 mg/l of EPS, respectively under its cultivation on whole or defatted milk or whey (0.5%, v/v) [34].

Thus, whey can be viewed as a potential cheap substrate for EPS synthesis, although only for a limited range of microorganisms able to assimilate lactose. The value for EPS synthesis on native whey is far lower than on traditional carbohydrate substrates, although pre-treatment of whey (deproteinisation, hydrolysis) allows to raise the efficiency of substrate biotransformation into EPS in several times. It is quite evident that nowadays it is an urgent task to create gene-engineered strains-producers of EPS, able to utilize lactose.

Crude glycerol

In our previous review [16] we stated that yearly increase in biodiesel production will be 8–10% and the market is forecasted to reach 37 billion gallons in 2016 (around 140 million tons). By May 2013, the amounts of biodiesel production in the countries of European Union reached 111 million gallons [16].

For every 100 l of biodiesel, almost 10 l of crude glycerol are produced (so-called glycerol fraction) [16]. The glycerol fraction contains a lot of impurities, which makes it impossible to use in many traditional fields of glycerol application [35]. Let us note that, due to increased alkalinity and methanol content, crude glycerol's storage and utilization are major environmental problem.

It has been established [36] that crude glycerol can be used as a substrate for xanthan synthesize by strain *X. campestris mangiferaeindica*e 2103. The concentration of synthesized xanthan was 7.23 g/l under producer's cultivation in the medium with 2% (v/v) of glycerol and 0.01% of urea. The viscosity of this EPS was 1.5-folds higher than synthesized in analogous conditions on sucrose.

Freitas et al. [37] established that strain *Pseudomonas oleovorans* NRRLB-14682 synthesizes high-molecular EPS under its cultivation on purified and crude glycerol. The highest synthesis values (concentration — 12.18g/l, productivity — 3.85 g/l/day, yield per substrate — 0.36 g/g) were reached when the bacteria were cultivated on crude glycerol (50 g/l), and when the producer was grown on the purified substrate, the values were 11.82 g/l, 2.00 g/l/day and 0.28 g/g, respectively.

Currently, there are but single reports of microbial EPS synthesis on the biodiesel production waste, although during the last decade it was established that crude glycerol can be used to synthesized other microbial-derived products (mono- and dicarbonic alcohols, polyols, organic acids, compounds with complex structure — polyhydroxoalkanoates, surfactants, cephalosporin, cyanocobalamin) [16]. Due to the presence of potential inhibitors in crude glycerol (methanol, Na and K salts), the efficiency of technologies of most microbial synthesis on this substrate is lower than on purified glycerol. The content of microbial polysaccharides synthesized on crude glycerol is also lower than on traditional carbohydrate substrates. However, the necessity of toxic industrial waste of biodiesel production compensates the relatively low values of final product synthesis. Besides that, today, bearing in mind the volumes of crude glycerol produced alongside biodiesel production, preference is given to microbial technologies which permit to biosynthesis of valuable metabolites in media with maximally possible waste content.

Oil- and fat-containing waste

The yearly world production of vegetable oils is around 160 million tons. The four most common ones (sunflower, soya, rapeseed and palm) account for 90% of all trade and 75% of all production volume [http://www.eurasiancommission.org]. The processing of plant materiel produces large amounts of waste. However, waste (fried) oil is the cheapest oil-containing waste accumulates not only in food factories, but also in catering facilities. For example, only in Europe 1.85–2.65 million liters of fried vegetable oil are produced daily [38]. One of the ways of oils utilization is using them as substrates for synthesis of valuable products, for example polysaccharides.

Salvador et al. [39] studied the ability of the fungus *Pleurotus ostreatus* FPO-1001 to synthesize EPS on waste sunflower oil. When substrate concentration was 10 g/l, strain FPO-1001 produced 0.8 g/l of the polysaccharide.

Strain *Cellulomonas flavigena* UNP3 on the 8th day of cultivation in the medium with 1% of peanut oil synthesized 1 g/l of polysaccharide which had high emulsifying properties [40].

Olive oil is produced on the plants with a lot of wastewater. These waste are quite rich in fat acids (oleic, linolenic, palmitic, arachidic) and other nutrients from olives, and so can be used for microbial EPS synthesis [25, 41]. Fresh wastewaters are phytotoxic due to the presence of phenolic compounds, which is why the problem of utilizing these waste is investigated in many countries [41].

There is a review [25] providing data on xanthan synthesis by *X. campestris* NRRL B-1459 S4LII in a medium with wastewater of olive oil production. At the substrate concentration of 20% (v/v) strain NRRL B-1459 S4LII synthesized 7 g/l of polysaccharide.

There is a report [42] on the possibility of using petroleum-containing waters after the washing of drilling equipment, and fat-containing wastewater from fish-processing factories to synthesize EPS by *Rhizobium*

leguminosarum ATCC 10004. The EPS concentration peaked at 42.4 g/l at 96th hour under strain cultivation in a medium with a mixture of both wastewater (−50%, v/v).

Acinetobacter sp. DR1 was grown in a medium with 1−3% (v/v) of motor oil [43]. It was established that the producer synthesized around 780 mg EPS/g biomass at motor oil concentration of 2%. Using higher concentrations of motor oil inhibited cells growth due to the presence of toxins in it.

Our studies showed that to synthesize ethapolan, a complex exopolysaccharide preparation (producer *Acinetobacter* sp. 12S, registered in the Depository of microorganisms of Institute of Microbiology and Virology of NAS of Ukraine under the number of IMB B-7005), wide range of mono-, and mixed C_2−C_6-substrates (ethanol, acetate, propanol, pyruvate, C_4-dicarboxylic acids, carbohydrates — mono- and disaccharides, starch, molasses etc.) can be used [8].

Further research showed that it was possible to use sunflower oil as carbon and energy source to synthesize ethapolan [44−46]. Earlier it was established [46] that the optimal concentration of refined oil for EPS synthesis was 5%. Table 1 shows the values for ethapolan synthesis on a medium containing the same concentrations of unrefined and waste sunflower oils. It was found that the maximum amount of ethapolan (14.4−15.5 g/l) was observed under strain IMB B-7005 cultivation on unrefined and waste after meat frying oils with using inoculum grown on refined oil.

Oil-containing substrates have another advantage beyond low costs. In most cases, there is no need to add nutrients or growth factors to the culture medium, since oil-containing materiel has a sizable amount of vitally important nutrients. Besides that, such substrates do not require sterilization, are cheap and available in large quantities. As for the disadvantages of the oil-containing waste, they are hydrophobic and insoluble in water, which substantially complicates the technological process. Also, fried oils, depending on frying characteristics, number of times in use and the kind of prepared foods, can contain toxic admixtures. Note, that EPS synthesis values for such substrates are lower than for carbohydrates.

Waste of agro-industrial complex
Beet pulp hydrolysate. Besides molasses, another waste of sugar production is pulp, which consists mostly of cellulose, hemicellulose, pectin, mono- and disaccharides [47]. In countries with developed animal husbandry it is used to feed cattle, in other ones it goes to fertilize fields. However, pulp can be used as a renewable resource in biotechnological processes.

The literature has a few reports of xanthan synthesis on this substrate [25, 46]. The pulp was pre-hydrolyzed with 1% of HCl, filtered (after which the pH was adjusted to 7.0) and sterilized at 121 °C. The obtained solution was diluted down to 2% (by glucose) to use for cultivation. The medium was supplemented with 2.2−5 g/l of citric acid as a chelating agent and an additional carbon source. When the medium with pulp hydrolysate was augmented with 4.4 g/l of citric acid, *X.campestris* ATCC 15206 synthesized 20.82 g/l of xanthan [47].

Table 1. **Ethapolan synthesis on oil-containing substrates depending on inoculate preparation**

Sunflower oil in the medium for:		EPS, g/l	g EPS/ g biomass
Inoculate preparation	EPS biosynthesis		
Refined	Refined (control)	13.1 ± 0.66	7.5 ± 0.38
	Unrefined	15.5 ± 0.78*	4.9 ± 0.25*
	Waste after meat frying	14.4 ± 0.72*	6.3 ± 0.32*
	Waste after potato frying	4.2 ± 0.21*	3.3 ± 0.17*
Unrefined	Unrefined	10.7 ± 0.54*	3.8 ± 0.19*
Waste after meat frying	Waste after meat frying	9.7 ± 0.49*	5.9 ± 0.29*
Waste after potato frying	Waste after potato frying	8.1 ± 0.41*	4.3 ± 0.22*

Note: * — $P \leq 0.05$ relative to control (EPS content and EPS-synthesizing ability under strain cultivation on refined oil).

Potato peels. Spoiled potatoes, potato peels after crisps production or any other way of processing potatoes can serve as substrates since they are rich in carbohydrates and other nutrients, and are available in large quantities.

Vidhyalakshmi et al. [48] report about xanthan synthesis by *Xanthomonas citri* MTCC 2286 and three isolated strains (*X. campestries, X. oryzae, X. musacearum*) with using potato peels as the substrate. So, in the process of cultivation in the medium with extract obtained from shredded peels (50 g/l), the producers synthesized 2.9, 2.87, 1.5, and 0.5 g/l of EPS, respectively.

Starch grits. The fungi *Ganoderma applanatum* 1572 and *G. lucidum* 1621 were able to synthesize EPS (13.9 and 7.1 g/l, respectively) if grown in a medium with 20.0 g/l of starch grits (waste of Public joint-stock company Kremnyansky Starch Plant). The content of the native grits (%, w/w): starch — 76.3; protein — 15.6; lipids — 1.3; endopolysaccharides — 5.2; ash — 1.6) [32].

Grape marc. There are but few reports with data on polysaccharide synthesis on grape marc (pomace) — the solid mass which is left after the juice separating off from berries in the production of wines and juices. Of more than 10 million tons of pomace (seeds, skins, stems) yearly accumulated, only an insignificant part is used to feed cattle. Most of it is thrown out, creating environmental problems [25].

Pullulan is known to be synthesized by strain *A. pullulans* NRRLY-6220 on grape pomace [25]. To use the pomace as a substrate they it was treated with water at 65–70 °C, the extract was diluted down to 7.4% (by glucose). When the producer was grown on such substrate, the maximal amount of the synthesized polysaccharide was 22.3 g/l at the seventh day of cultivation [25].

According to [49], EPS (14.68 g/l) was synthesized by a mutant strain *Alternaria alternate* MIS4 when grown on extract of spoilt grapes (carbohydrate content — 150 g/l).

Juice of spoilt fruits. At juice and other factories, lots of waste are produced (skins, seeds), and some of the fruit are spoilt and unusable for further procession [50]. Due to high carbohydrate content, such waste can be used in biotechnological facilities as growth substrates.

There are data [50] on cellulose synthesis by strain *Gluconacetobacter xylinus* ATCC 53582 on a medium containing juice of spoilt fruits (plums, grapes, green pineapples and apples). To minced fruits (250 g), 400 ml of distillate water were added and centrifuged. The supernatant at 60% (by carbohydrates) was used as substrate. On the fourth day of cultivation, strain ATCC 53582 synthesized up to 60 mg/ml of cellulose.

Cocoa nutshells extract. Cellulose (19.9%), hemicellulose (68.7%), lignin (30.1%) are the main components of cocoa shells which can be transformed into simple carbohydrates by hydrolysis. The increase in cocoa juice production is accompanied by 6.7 million tons' worth of cocoa shells per year [51].

Nery et al. [51] established the ability of *X. campestries* 1866 to synthesize xanthan (10.3 g/l) in a medium with 80 g/l (by carbohydrates) of cocoa shell extract.

Extract of spoilt date fruit and their remains. Waste, mostly dates which have dropped down before maturing, seeds of fruit and pressed puree (processed dates) accumulate in large amounts and constitute an urgent environmental problem. Only in Tunisia there are 100000 tones of such waste produced every year [52].

The study reports about using dates extract to synthesize xanthan. At the concentration of 84.68 g/l (by carbohydrates) *X. campestris* NRRL B-1459 produced 43.35 g/l of EPS. Curdlan producent *Rhizobium radiobacter* ATCC 6466 was cultivated on a medium containing extract of dates as the main component (120 g/l by glucose). In such conditions, the maximal concentration of synthesized polysaccharide was 22.83 g/l.

Water extract of cabbage

Cabbage is the native habitat of bacteria belonging to the species *X. campestris*, which is why it can be used as carbon source for xanthan production. During cultivation of *X. campestris* NCPPB 528 on a medium containing water extract of cabbage, the producer synthesized 9.8 g/l of EPS. The extract was prepared by grinding 1kg of cabbage and adding 1 l of water, mixing until suspension was formed, and then straining off the roughage [53].

Hydrolysate of rice bran. Sirajunnisa et al. [22] established the ability of *B. subtilis* WCS36 to synthesize up to 2.14 g/l of polysaccharide in a medium with hydrolysate of fine rice bran as its main component (5%, v/v).

It is known from the literature [25] that EPS can be synthesized on rice bran hydrolysate by nitrogen-fixing bacteria *Sinorhizobium meliloti* MTCC 100. 11.8 g/l of polysaccharide were synthesized under strain

MTCC 100 cultivation in a medium with this hydrolysate (20 %, v/v).

Minced chicken feathers. It was established [54] that *Morchella esculenta* ATCC 10968 can synthesize EPS on a mixture of glucose (40 g/l) and minced hen feathers (10 g/l), which contains 90% of protein. Under these conditions, the producer synthesized about 4.6 g/l of polysaccharide.

The disadvantages of using lignin-containing waste for EPS synthesis (pulp, grape pomace, spoilt nuts, bran) are their insolubility and the fact that most producers lack enzymes necessary to hydrolyze cellulose, which necessitates pre-hydrolysis of such substrates. The extracts (juices) of fruits and vegetables do not have these disadvantages, but preparing them also requires additional steps to use them for microbial EPS synthesis. For some waste of agricultural industry, the target product concentration is almost as large as the values for traditional carbohydrate substrates (up to 40 g/l), but usually it is several times lower.

Other substrates

Extract of shrimp shells is a cheap waste usable to synthesize xanthan. The carapaces contain protein (8.1%), lipids (0.8%) and carbohydrates (1.4%) [20]. Strain *X. campestris* 1182 in a medium with 10% (v/v) of the extract synthesized 4.64 g/l of xanthan [20].

Carbon dioxide (CO_2) is non-inflammable, reductive substance which is contained in the atmosphere in excess and can serve as a carbon source for autotrophic microorganisms. Its biotransformation into valuable metabolites enhances the progress of green biotechnology and positively influences the CO_2 balance in nature. Microalgae are able to fix carbon dioxide and biotransform it into polysaccharides [25].

There are several research reports on EPS synthesis by *Botryococcus braunii* microalgae. For example, under *B. braunii* LB 572 strain cultivation in airlifting bioreactor with fresh potable water, 1.6 g/l of exopolysaccharides were synthesized. This very strain synthesized up to 2−3 g/l of EPS in increased salinity. *Porphyridium cruentum* GUMACC 25 synthesizes 543.1 mg/l of EPS in pH 8.0 and light intensity 7 100 lx [25].

Bark hydrolyzate of carob trees. Fruits and seeds of the carob tree *Ceratonia siliqua* L. are used in food industry. The tree's bark is reportedly used as a substrate in bioethanol production [25]. That article also includes data on pullulan production by *A. pullulans* SU-M18 strain on bark extract of *Ceratonia siliqua* L. This strain synthesized 2.16 g/l/day of EPS when such extract was used in concentration of 25 g/l (expressed as glucose). Under cultivation in medium with extract of shredded bark of carob tree, *Leuconostoc mesenteroides* NRRL B512 produced 8.56 g/l of dextran.

Shrimp shells and carob tree bark in exopolysaccharide biosynthesis necessitate the introduction of additional extraction and hydrolysis stages respectively in the technological scheme of obtaining final product. The indicator values of EPS synthesis on these substrates are several times lower than on traditional substrates. Carbon dioxide is expedient in EPS production though the present relevant technologies are unlikely to be industrialized due to the lack of highly productive autotrophic producers.

Data on EPS synthesis on industrial waste are summarized in Table 2.

Thus, according to analysis of recent reports, there are inexpensive and present in large amounts industrial waste (milk whey, crude glycerol, oil-containing and agro-industrial waste) that can be used in biotechnological production of microbial polysaccharides. Implementation of industrial waste as substrates aside from their availability has technological, economical and ecological advantages:

− crude glycerol and waste vegetable oils do not need sterilization or anti-floam agents during cultivation which significantly reduces technological costs;

− agro-industrial waste contain different nutrients that can activate the microorganisms growth and EPS synthesis;

− crude glycerol and fried vegetable oils are ecologically dangerous and toxic. Their emissions to the environment are not regulated, hence recycling such waste is extremely topical today.

Simultaneously, compared to the more expensive traditional carbohydrate substrates for the EPS biosynthesis, industrial waste are also associated with drawbacks, the main of which is low efficiency of target product bioconversion. The data on advantages and disadvantages of industrial waste implementation for exopolysaccharide production are summarized in Table 3.

Table 2. **Alternative carbon sources for synthesis of microbial exopolysaccharides**

Producer	Substrate	Substrate concentration	EPS, g/l	Refe-rences
M. rouxii NRRL 1894	Molasses	30 g/l	7.2	[19]
X. campestris ATCC 1395	« – »	175 g/l	53	[20]
H. smyrniensis AAD6	« – »	30 g/l	12.4	[21]
B. subtilis WCS36	« – »	2%	4.86	[22]
Z. mobilis ATCC 31821	« – »	250 g/l	2.53	[25]
S. paucimobilis ATCC-31461	« – »	2%	13.82	[25]
A.pullulans MTCC 1991	« – »	50 g/l	27.3	[24]
B. subtilis 168	« – »	2.4%	4.9	[23]
X. campestris pv.*campestris*	« – »	5%	19.8	[18]
S. rolfsii MTCC 2156	Sugar syrup	120 g/l	23.78	[25]
Z. mobilis ATCC 31821	« – »	250 g/l	15.5	[25]
X. campestris PTCC 1473	Milk whey	30 g/l (by lactose)	16.5	[28]
X. campestris XLM 1521	« – »	50% (v/v)	12	[20]
X. campestris ATCC 13951	« – »	43 g/l (by lactose)	28	[27]
S. paucimobilis ATCC 31461	« – »	52 g/l (by lactose)	7.0	[29]
G. applanatum 1572	« – »	60 g/l (by lactose)	9.1	[32]
G. lucidum 1621	« – »	60 g/l (by lactose)	10	[32]
S. thermophilus BN1	« – »	0.5% (v/v)	375 mg/l	[34]
Z. profunda SM-A87	« – »	60.9% (v/v)	12.1	[33]
X. campestris mangiferaeindicae 2103	Crude glycerol	2% (v/v)	7.23	[36]
P.oleovorans NRRLB-14682	« – »	50 g/l	12.2	[37]
X. campestris NRRL B-1459 S4LII	Wastewater of olive oil production	20% (v/v)	7.0	[25]
C. flavigena UNP3	Peanat oil	1% (v/v)	1.0	[40]
P.ostreatus FPO-1001	Waste of sunflower oil production	10 g/l	0.8	[39]
Acinetobacter sp. DR1	Engine oil	2% (v/v)	5 g EPS per g of biomass	[43]
X.campestris ATCC 15206	Pulp hydrolysate	2% (by glucose)	20.8	[47]
X. citri MTCC 2286	Potato peel extract	50 g/l	2.9	[48]
G. applanatum 1572	Starch grits	20 g/l	13.9	[32]
G. lucidum 1621	« – »	20 g/l	7.1	[32]
A. pullulans NRRLY-6220	Grape marc extract	7,4% (by glucose)	22.3	[25]
Alternaria alternate MIS4	« – »	150 g/l (by carbohydrates)	14.68	[49]
G. xylinus ATCC 53582	Juice of spoiled fruits	60% (by carbohydrates)	60 g/l	[50]
X.campestries 1866	Coconut shell extract	80 g/l (by carbohydrates)	10.3	[51]
X. campestris NRRL B-1459	Extract of spoiled dates and their remains	84.68 g/l (by carbohydrates)	43.35	[52]
R. radiobacter ATCC 6466	« – »	120 g/l (by glucose)	22.83	[52]
X. campestris NCPPB 528	Water extract of cabbage (1 kg cabbage + 1 l water)		9.8	[53]
B. subtilis WCS36.	Hydrolyzed rice bran	5% (v/v)	2.14	[22]
S. meliloti MTCC 100	« – »	20% (v/v)	11.8	[25]
X. campestris 1182	Shrimp shell extract	10% (v/v)	4.64	[20]
M. esculenta ATCC 10968	Crushed chicken feathers	10 g/l + 40 g/l glucose	4.6	[54]
A. pullulans SU-M18	Hydrolisate of carob tree bark	25 g/l (by carbohydrates)	2.16 g/l per day	[25]

Table 3. **The summarized characterization of industrial wastes,
used for the biosynthesis of exopolysaccharides**

Industrial wastes	Advantages	Drawbacks	EPS concentration compared to traditional carbohydrate substrates
1	2	3	4
Molasses	High saccharose content, availability, present in large amounts, hydrophilic substrate. The presence of organic nitrogen allows to reduce the content of nitrogen nutrition source in the culture medium	Contain potential inhibitors of microbial growth (betaine, Fe and Zn cations). Require preliminary hydrolysis (if the EPS producers lack invertase)	Almost identical
Milk whey	Hydrophilic, relatively inexpensive, always present in large amounts	Can be assimilated by limited range of microorganisms, needs pretreatment (for example, deproteinization). GM-strains are required	Native whey provides approx. ten times lower EPS concentration. Pretreated whey allows for several times lower EPS concentration.
Crude glycerol	Hydrophilic, requires no sterilization, inexpensive, available in very large amounts	Contains toxic admixtures, frequently needs preliminary filtering	Several times lower
Waste oils	Require no sterilization, inexpensive, contain nutrients, available in large amounts	Hydrophobic, insoluble in water, can contain toxic admixtures depending on the oil circulation multiplicity	Lower
Agro-industrial waste	Inexpensive, contain nutrients, always available in large amounts	Require preliminary hydrolysis	Usually lower
Carbon dioxide	Available in large amounts	Uses autotrophic microorganisms as producers	Ten times lower

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ОТРИМАННЯ ЕКЗОПОЛІСАХАРИДІВ ІЗ ПРОМИСЛОВИХ ВІДХОДІВ

Т. П. Пирог
М. О. Івахнюк
А. А. Вороненко

Національний університет харчових технологій, Київ, Україна

E-mail: tapirog@nuft.edu.ua

Наведено дані літератури і результати власних досліджень авторів щодо синтезу мікробних екзополісахаридів на різних промислових відходах (харчової промисловості, сільськогосподарського сектору, виробництва біодизеля тощо). Використання промислових відходів для отримання екзополісахаридів дасть змогу не лише вирішити проблему накопичення вторинної сировини, а й зменшити витрати на біосинтез практично цінних метаболітів. Зроблено висновок про те, що застосування деяких відходів порівняно з традиційними вуглеводними субстратами, окрім екологічних, має низку технологічних переваг: наявність ростових факторів, відсутність потреби у піногаснику та стерилізації субстрату.

Ключові слова: екзополісахариди, промислові відходи, інтенсифікація біосинтезу.

ПОЛУЧЕНИЕ ЭКЗОПОЛИСАХАРИДОВ ИЗ ПРОМЫШЛЕННЫХ ОТХОДОВ

Т. П. Пирог
Н. А. Ивахнюк
А. А. Вороненко

Национальный университет пищевых технологий, Киев, Украина

E-mail: tapirog@nuft.edu.ua

Представлены данные литературы и результаты собственных исследований авторов, касающиеся синтеза микробных экзополисахаридов на различных промышленных отходах (пищевой промышленности, сельскохозяйственного сектора, производства биодизеля и др.). Использование промышленных отходов для получения экзополисахаридов позволит не только решить проблему накопления вторичного сырья, но и снизить затраты на биосинтез практически ценных метаболитов. Сделан вывод о том, что применение некоторых отходов по сравнению с традиционными углеводными субстратами, кроме экологических, имеет ряд технологических преимуществ: наличие в составе ростовых факторов, отсутствие необходимости в пеногасителе и стерилизации субстрата.

Ключевые слова: экзополисахариды, промышленные отходы, интенсификация биосинтеза.