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Effect of some factors on the production of curde exopolysaccharides with antioxedant activity from marine bacteria

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Abstract

It is possible to use marine microbial polysaccharides as antioxidants in a variety of sectors because of their unique properties. A total of 12 bacterial isolates were gathered from a variety of coastal habitats for further study. These bacteria were tested for exopolysaccharides (EPSs) synthesis, with the top bacterial isolate ES12 producing 6.8 g/l. DPPH free radical scavenging activity was used to assess antioxidant activity, with ES12 (97.20 percent) and ES6 (19.81 percent) being the highest and lowest, respectively, whereas five EPSs did not demonstrate this activity. The maximum yield of EPS was 8.2 g/l and cell dry was (4.5 g/l) at 40°C after three days, RPM 120, pH 7, peptone as nitrogen sources, and sucrose with (20 g/l) as a carbon source for isolate (ES12) in the investigation.

Keywords: Exopolysaccharides, Production, Antioxidant, Marine, Bacteria

1. Introduction

In plants, animals, and microorganisms polysaccharides are found in abundance. Because they are macromolecular macromolecules like proteins and polynucleotides, like polysaccharides, the immune system relies on them to communicate with and adhere to other cells as well as recognise molecules [1].

Products from biopharmaceutical, functional, food additive, and polysaccharide vaccine producers are all utilising EPS as active components. Antioxidants, antistaling agents, and emulsifiers are all functions of these EPSs, in addition to their role as flocculants for microbes [2].

Oxidative stress arises when the rate at which reactive oxygen species (ROS) are generated is outpacing the body's ability to remove or repair the damage produced by the ROS [3].

Oxygen species (ROS) and free radicals produced by oxygen have recently been found to have a wide range of pathogenic consequences (such as DNA damage, carcinogenesis, and cellular degeneration), and may also contribute to the onset of many chronic illnesses like Alzheimer's and rheumatoid arthritis (RA) [4,5]. A wide range of therapeutic scenarios were used to comprehensively evaluate natural biomaterials. synthetic antioxidants cannot be widely used because of their carcinogenicity [6]. Natural antioxidants that are safe for humans must be produced for this reason. There has been a lot of interest in novel natural antioxidants in recent years in both science and medicine [7].

Among the several naturally occurring 3 anti-oxidants, polysaccharides have been shown to exhibit significant levels of antioxidant activity [8,9]. They are excellent anti-oxidants and cancer immunotherapies because they have little side effects, such as cytotoxicity, making them suitable. According to Osuntoki and Korie [10] (2009), [11].

2. Materials and Methods

The gathering of marine samples and the separation of bacteria

Samples from Red Sea marine sediment sources were gathered from several places. Media (gm/l) was used to isolate bacteria. pH 7.0-7.4 Glucose 20, $CaCO_3$,1.0, NH_4NO_3,0.8, KH_2PO_4, 0.05; K_2HPO_4, 0.6, $MgSO_4$. $7H_2O$, 0.05; $MnSO_4$. $4H_2O$, 0.1; Yeast extract 0,1 and agar 15,0 [12]. As Hayakawa and Nonomura [13] recommended, the medium was diluted in sea water to 1 L using the serial diluting process (1987).

EPSs production from liquid culture is being screened for.

Bacterial isolates were tested for the formation of EPSs in a liquid production medium. A 250 mL beaker was used to inoculate the purified isolates into a screening medium containing peptone 5.0 percent, meat extract 3.0 percent, $FeSO_4$ 0.01 g/l, and agar 15, g/l, dissolved in seawater that had been completed to 1 litre for distal water with an adjusted pH of 7 [14]. Afterward, the culture media was centrifuged twice at 5000 rpm for 10 minutes to remove protein from the supernatant, followed by an overnight incubation at 4 °C. To get the clear solution's pH up to 7, we employed a NaOH solution. The supernatant was diluted with four litres of ethanol 95 percent and kept overnight at 4°C. After centrifugation at 5000 rpm for 20 minutes, the EPS precipitation was separated by washing twice with acetone and drying with ether [15].

An evaluation of the anti-oxidant properties

Antioxidant effectiveness of several bacterial crude exopolysaccharides was examined at various intervals. According to Brand-Williams et al.,[16] the free radical-scavenging activity (RSA) was measured by measuring the

discolouration of the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical solution at 517 nm (1995).
The following formulae were used to assess scavenging activity:

Scavenging ability (%) = ($A_{517 \text{ of control}}$ - $A_{517 \text{ of sample}}$ / $A_{517 \text{ of control}}$) ×100.

Optimization of the EPSs manufacturing medium is the fourth step.

Optimizing the media components necessary to maximise EPS output. EPS production was highest in broths that included peptone (5.0), meat extract (3.0), FeSO4 (0.01), agar (15 g/l), and 1 litre of sea water, all of which were dissolved in 750 millilitres of sea water. Later, different incubation times (2, 3, 4, 5 and 6 days), pH levels (5, 6, 7, 8 and 9) adjusted with 1 N HCl or 1 N NaOH, temperatures (25, 30, 35, 40, 45 and 50 C), RPM (static, 50, 100, 120, 150 and 200), inoculum sizes (250, 500, 750 and 1000l) were all examined as well as the effects of various medium components. Incorporation of various additional carbon sources (glucose at 1 percent by weight), sucrose concentrations (at 10, 15, 20, 25 and 30 grammes per litre), and organic nitrogen sources (at arabinose and mannose at 1 percent by weight) are all used, as are various concentrations of sucrose and a variety of organic nitrogen sources (yeast extract, malt extract, peptone, beef extract, potassium nitrate, ammonium oxalate, ammonium molybidate and ammonium sulfate).

3. Results and Discusion

Different bacterial isolates were isolated, and their cell dry weight and exopolysaccharides were determined.

Many different areas, from the coast to the deeper channels, are affected by maritime climate, and each has its own unique geochemical and physical characteristics that contribute to the world's greatest biodiversity [17]. The Red Sea included 12 different bacterial strains, all of which were absolutely unrelated to one another. In order to look for the development of exopolysaccharides, bacteria were grown for three days. Dried to a consistent weight at 80 degrees Celsius. To prepare the crude EPSs, five volumes of ethanol were used to precipitate the material, which was then cleaned in acetone and dried in ether. The dry weight, EPS, and productivity of 12 marine bacterial isolates were shown in Table 1. While the greatest bacterial isolate (ES12) generated EPS 6.80 gm/L from 3.85 gm/L cell dry weight, the least (ES2) produced EPS 1.47 gm/L and had a Productivity of 93%.

Table (1) Cell dry weight (CDW), EPS and productivity of marine bacterial isolates

Isolate	CDW (gm/L)	EPS (gm/L)	Productivity (%)
ES 1	1.92	2.65	138
ES 2	1.57	1.47	93
ES 3	1.95	3.15	161
ES 4	2.01	3.54	176
ES 5	2.1	3.89	185
ES 6	2.47	2.15	87
ES 7	1.85	3.14	169
ES 8	2.31	1.54	66
ES 9	2.10	1.96	93
ES 10	3.58	5.47	152
ES 11	3.95	4.98	126
ES 12	3.85	6.80	176

Assessment of the antioxidant activity of DPPH

As free radical scavengers and antioxidants, polysaccharides are essential to maintaining a healthy balance in living organisms when exposed to oxidative harm [18]. It was thus decided to conduct an anti-oxidant screening of twelve different EPSs. Antioxidant activity of these EPSs is shown in Table (2) with the highest one being ES12 (97.20%) at 120 minutes and the lowest being ES6 (19.81%) at 90 minutes. While there are five EPSs that do not have any antioxidant activity. At varied dosages (1, 2, 3 and 4 mg) and different times (30, 60, 90 and 120 minutes), the antioxidant activity of marine bacterial ES 12 was shown in Table 3 to be strong (98.39 percent) after 120 minutes.

Table (2) DPPH free radical scavenging activity (%) for marine bacterial EPS at different periods.

Isolate no.	DPPH free radical scavenging activity (%)			
	30 min	60 min	90 min	120 min
ES 1	0.0	0.0	0.0	0.0
ES 2	15.18	20.24	58.19	58.10
ES 3	51.81	67.54	75.25	79.21
ES 4	0.0	0.0	0.0	0.0

ES 5	0.0	0.0	0.0	0.0
ES 6	10.75	11.12	17.25	19.81
ES 7	13.29	17.25	23.14	31.19
ES 8	25.02	31.09	35.27	39.91
ES 9	0.0	0.0	0.0	0.0
ES 10	0.0	0.0	0.0	0.0
ES 11	63.21	68.98	75.91	76.99
ES 12	87.95	94.09	96.18	97.20

Table (3) DPPH free radical scavenging activity (%) for ES 12

Concentration	DPPH free radical scavenging activity (%)			
(mg/ml)	30 min	60 min	90 min	120 min
1	70.68	75.64	80.12	90.23
2	73.01	78.91	80.14	91.81
3	85.11	87.33	89.09	94.20
4	89.98	92.60	95.92	98.39

EPS production is influenced by a wide range of factors.

Due to the limited quantity of each polymer in the natural marine environment, it is possible to establish an isolated bacterial strain in a laboratory to research EPS formation in the marine environment [19]. There is no one combination of growth conditions that assures high EPS yields since microorganisms vary widely in their capacity to absorb carbon and nitrogen sources, their mineral needs, temperature, and ideal pH. It's also possible to use a physiological control to tweak EPS's molecular weight, residue count, and branching pattern. I agree that the nutrients and environment (culture conditions) may impact the synthesis and quality of EPS from microorganisms, however this is not the only factor [20].

The maximal EPS production (7.0 g/l) at cell dry weight (4.2 g/l) was seen at 40°C, where the fermentation medium's incubation temperature is known to affect the development of isolate (ES12). EPS production is slowed down by temperature changes of any kind Fig. (1). ES12's productivity peaked at 7.1 gm/L cell dry weight (3.9 g/L) after three days of incubation before declining. EPS generation and incubation duration are controlled by the kind of organism, according to these data Fig. (2). RPM has an effect on the ES12 EPS output Fig. (3). A dry weight of 4.0 g/l and a maximum yield of 7.4 g/l were the results. As a result, EPS generation is influenced by the size of the bacterial inoculum in the fermentation medium. As can be seen in Figure 4, increasing the inoculum size decreases EPS production, whereas decreasing the cell dry weight causes the highest EPS production (7.8 g/l) at a cell dry weight of (4.1 g/l). ES12 had an impact on the amount of EPSs produced by pH. A pH increase or decrease led to a decrease in the amount of EPSs generated, however the maximum yield at pH 7 was 7.9 g/l and the cell dry weight was 4.6 g/l Fig. (5). According to [21] It was shown that the lowest cell dry weight was achieved with the maximum EPS concentration (10.8 g/l) while employing Pseudovibrio sp. strain 4MS 2020 at 40°C, pH 7, RPM 150, and yeast extract as a nitrogen source. Sucrose (20 g/l) was utilised as a carbon source. A considerable increase in EPS synthesis was also achieved by incorporating organic and inorganic nitrogen sources into the medium. Out of all the nitrogen sources tested, peptone supported the highest EPS production (8.6 g/l) with a cell dry weight (4.5 g/l). Variations in EPS production were seen across the various nitrogen sources studied Fig. (6). In terms of cell dry weight (0.5 g/l), ammonium oxalate was the least effective nitrogen source for the formation of EPSs (0.8 g/l). The generation of EPSs increased when different carbon sources were added to the medium at a 1% rate. Carbon sources other than glucose were all notable in their ability to generate considerable quantities of exopolysaccharides. Starch (0.9 g/l) was the least effective carbon source Fig. (7). As shown in Fig. (8), sucrose concentrations have an effect on the formation of EPS, which is consistent with previous studies. In this experiment, sucrose (20 g/l) produced the greatest EPS production (8.2 g/l) and the cell dry weight (4.3 g/l) was highest

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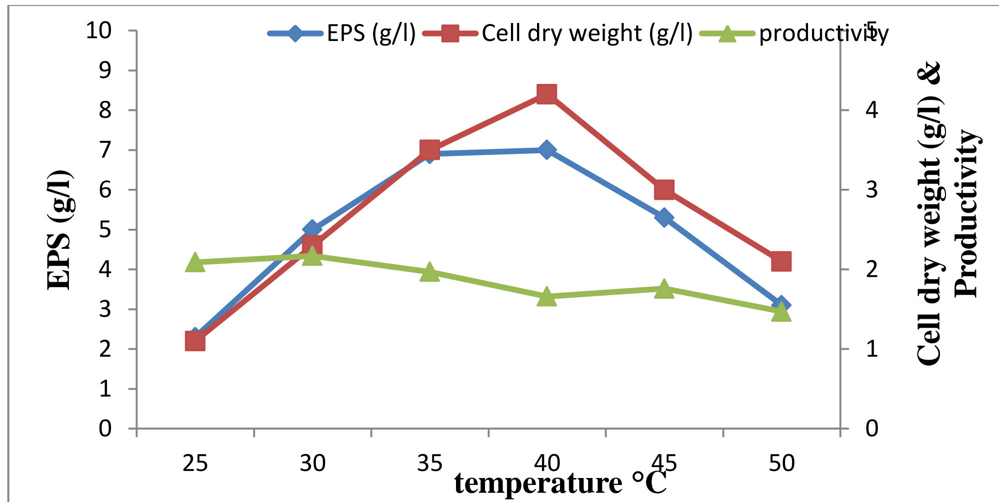

Fig. (1) effect of different temperature at production of ES 12

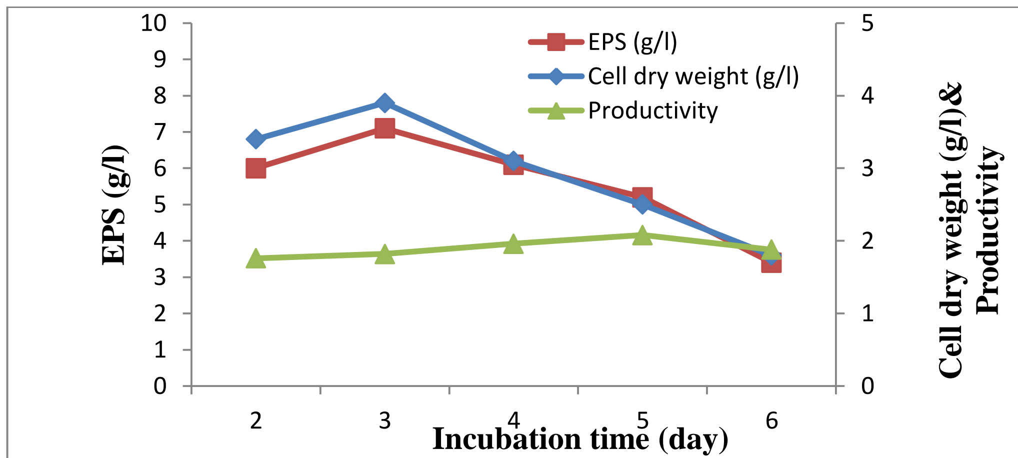

Fig. (2) effect of different incubation time at production of ES 12

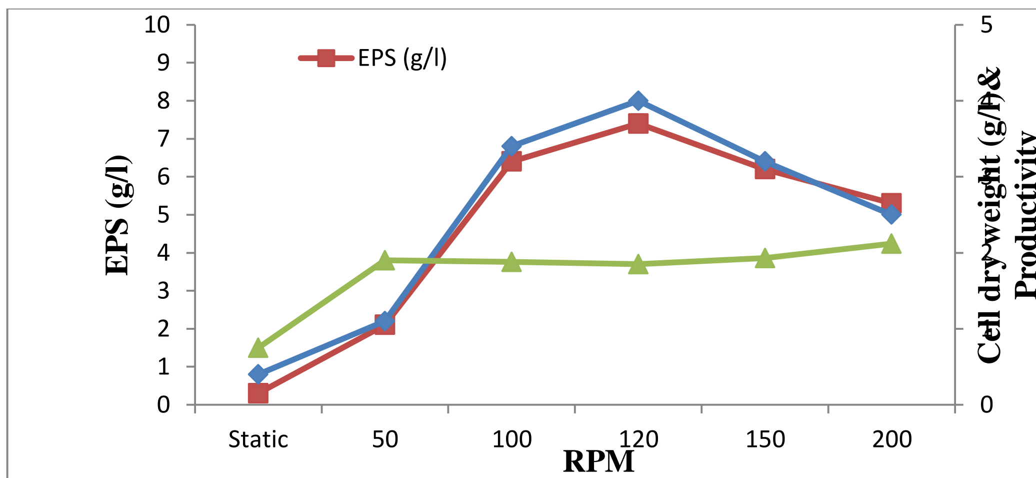

Fig. (3) effect of different RPM at production of ES 12

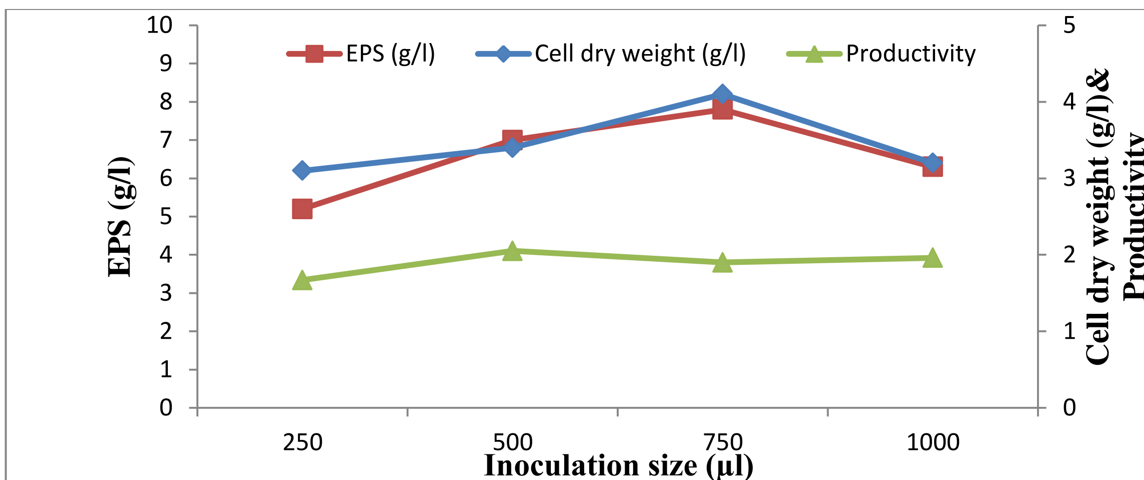

Fig. (4) effect of different Inoculation size at production of ES 12

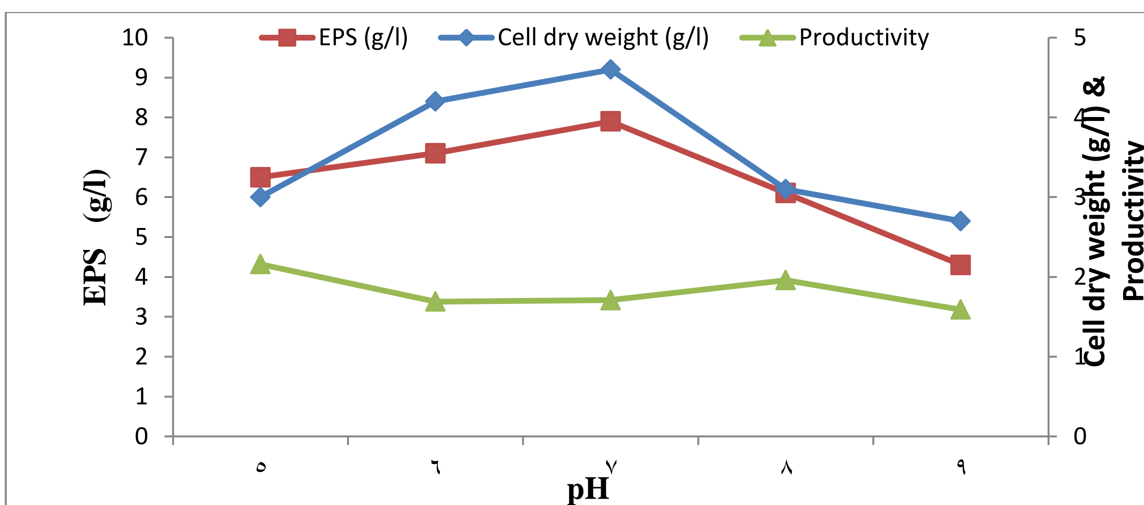

Fig. (5) effect of different pH at production of ES 12

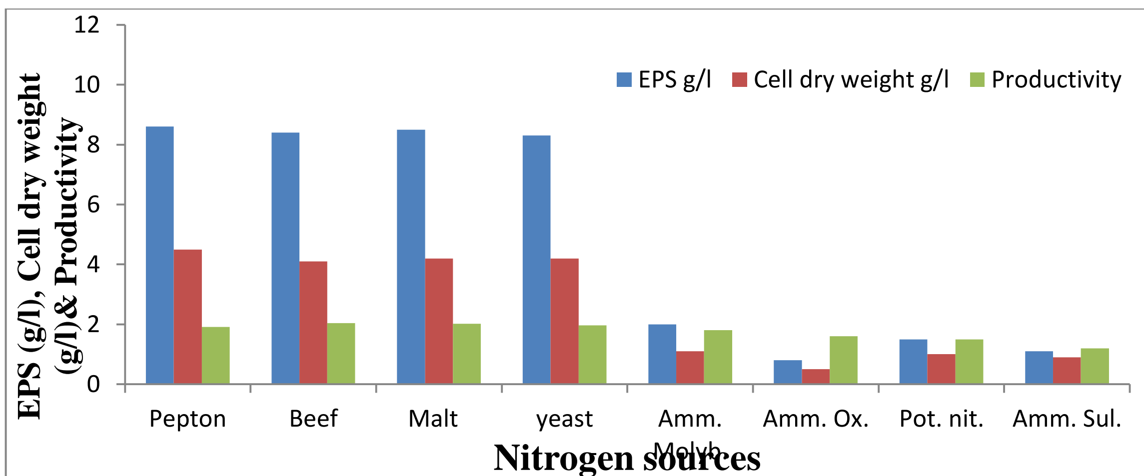

Fig. (6) effect of different nitrogen sources at production of ES 12

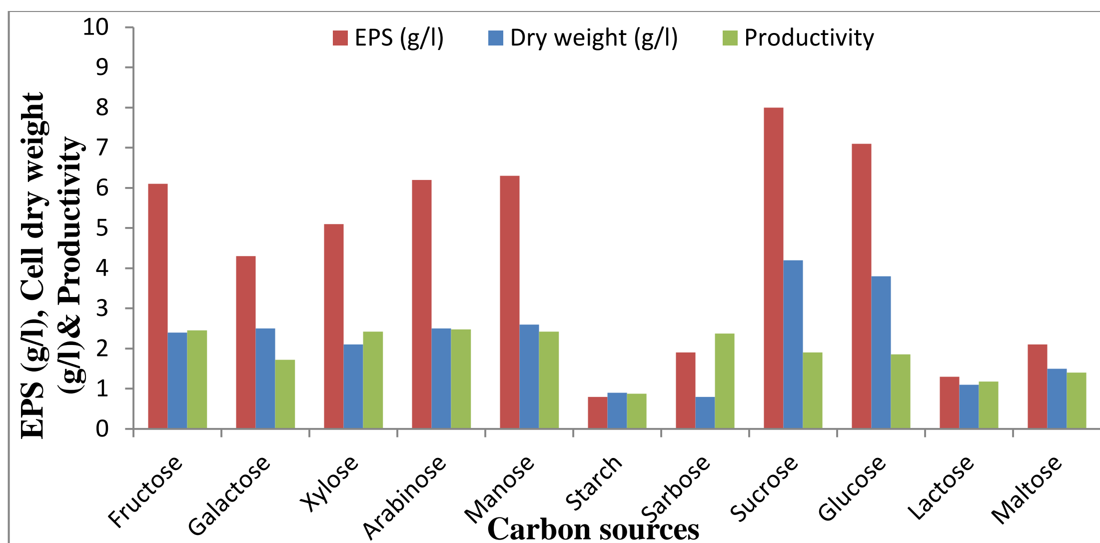

Fig. (7) effect of different carbon sources at production of ES 12

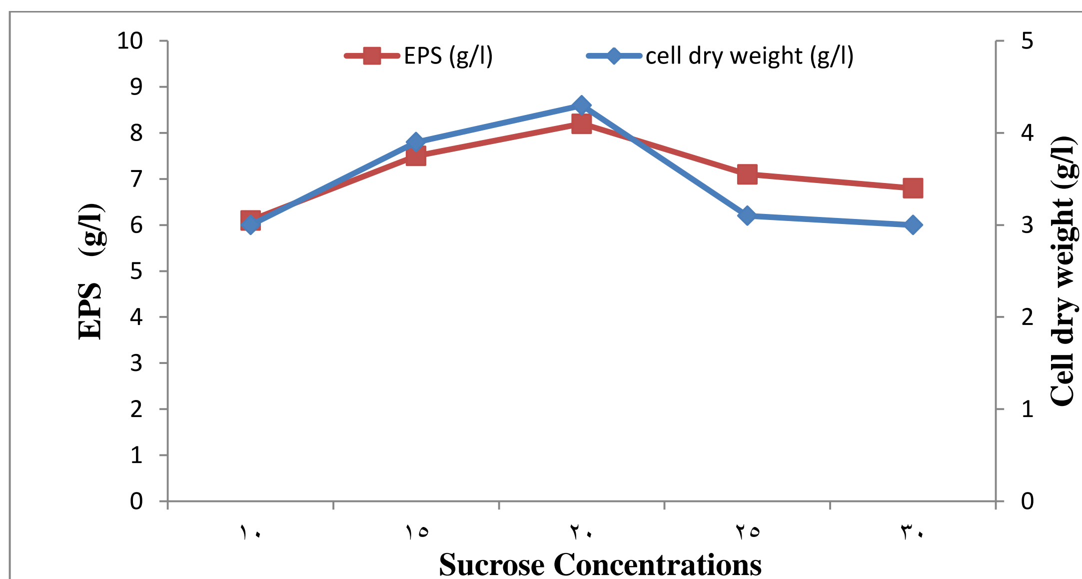

Fig. (8) effect of different sucrose concentration at production of ES 12

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