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production and Optimization of exopolysaccharides with antioxidant activity isolated from marine bacteria

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Abstract

Bacterial exopolysaccharides (EPSs) are biopolymers carbohydrate with high-molecular-weight which released often secreted extracellularly by bacteria. In the presented work, ten bacterial isolates were obtained from various marine samples. The bacterial isolate (BS6), was selected for its maximum productivity which reached (5.9 g/l) after being screened for exopolysaccharide (EPS) synthesis. As a result, the antioxidant activity of EPSs was determined by measuring their DPPH free radical scavenging activity. At 120 minutes, the best score was BS6 (98.40%), while the lowest score was BS1 (24.94%). On the other hand, Three EPSs, have no antioxidant action. The synthesis and production of EPS are influenced by a variety of variables. therefore, the effect of various factors on the production of EPSs by isolate (BS6) revealed that the maximum yield of EPS was (7.2 g/l) and cell dry was (3.9 g/l) when incubation temperature was 40°C for three days, RPM was 120, pH was 7, peptone was used as a nitrogen source, and sucrose was used as a carbon source.

Keywords: exopolysaccharides, production, antioxidant, marine, bacteria.

1.Introduction

Exopolysaccharides (EPS) are polymers with a high molecular weight that can be employed as bioadhesives, biosorbents, bioflocculants, stabilizes, gelling agents, and thickeners (1).

Exopolysaccharides, also known as extracellular pol ymeric substances (EPS), are a collection of biopolymers , primarily polysaccharides, produced by microorganisms such as bacteria, fungi, and microalgae and attached to t he cell surface as capsules or excreted in the extracellular environment as slime (2).

The EPS physiological role in microorganisms is connected to bacterial cell protection from extreme and harsh conditions such as biotic and/or abiotic stressors, as well as also for surface adherence and biofilm (3)

The exopolysaccharides production and composition can be slightly affected by culture and fermentation conditions (i.e., pH, carbon source, incubation time, temperature and) as well as strain-specific genetic pathways for EPS generation, which reflect variable conformational properties, sugar connections, and molecular mass (4).

People are becoming more aware of their health as their lifestyles improve. As a result, the demand for health-care items has increased significantly. People, on the other hand, examine not only the efficacy of these health-care goods, but also their source, giving preference to natural health-care items derived from animals or plants (5)

Antioxidants, as one example, are often employed in natural health care products. Antioxidants can scavenge reactive oxygen species, which can cause illnesses linked to oxidative stress (6). Despite the fact that synthetic antioxidants can prevent or mitigate the damage caused by reactive oxygen species, natural antioxidants, particularly those produced from plants, have seen an increase in demand in recent years due to the former's potential toxicological implications (7).

Natural polysaccharides with antioxidant activity have gained a lot of attention in natural medicine research and manufacture in recent years (8). In the current investigation, reducing ferric iron power, DPPH radical scavenging activity, chelating ferrous iron capacity, and hydroxyl radical scavenging activity assays were used to show that polysaccharide produced bungeanum has high antioxidant effects in vitro.

2. Materials And Methods

1. Collection of marine samples and isolation of bacteria

Marine samples were obtained from different locations at Red sea from. Bacterial isolates were isolated using the following media (gm/l.) Glucose, 20; $CaCO_3$,1.0; KH_2PO4, 0.05; NH_4NO_3, 0.8; K_2HPO_4, 0.6; 0.05; $MnSO_4$. $4H_2O$, $MgSO_4$. $7H_2O$, 0.1; Yeast extract, 0.1 and agar, 15.0 pH 7.0-7.4 **(Kim *et al*, 1998)**. Media were dissolved in 750 ml sea water completed to 1 L. by serial dilution method of **Hayakawa and Nonomura (1987)**.

2. Screening for EPSs production from liquid culture

In a liquid production medium, the bacterial isolates were tested for their ability to produce EPSs. The pure isolates were injected into a 250 mL flask containing 50 mL of screening producing medium contain meat extract 3.0, $FeSO_4$ 0.01, peptone 5.0, agar 15, g/l dissolved in 1 liter sea water: distal water (7.5:2.5), and the pH adjusted to 7 (11) and cultivated at 37°C, for 3 days at 120 rpm. After incubation, the culture medium was centrifuged at 5000 rpm for 10 minutes, the supernatant was mixed with 10% Trichloroacetic acid and stored at 4°C overnight before being centrifuged at 5000 rpm to remove protein. With NaOH solution, the pH of the clear

solution was changed to 7. The supernatant was diluted to four volumes with 95 percent ethanol and stored at 4°C overnight. The EPSs were separated by centrifugation at 5000 rpm for 20 minutes, washed twice with acetone, and ether dehydrated (12).

3. Assessment of antioxidant activity

At different intervals, the antioxidant activity of several bacterial crude exopolysaccharides was detected. The decoloration of a solution of DPPH (1,1-diphenyl-2-picrylhydrazyl) radical measured spectrophotometrically at 517 nm to determine the free radical scavenging activity (RSA) according to Brand-Williams (13).

Calculation of the scavenging activity was as follows:

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

4. Medium optimization for production of EPSs

maximum EPSs production via selected bacterial strain was achieved by optimization of media components such as (meat extract 3.0, peptone 5.0, $FeSO_4$ 0.01, agar 15, g/l dissolved in 1 liter sea water: distal water (7.5:2.5). Subsequently the physical conditions were also studied including different incubation times (2, 3, 4, 5 and 6 days), pH (5, 6, 7, 8 and 9) temperature (25, 30, 35, 40, 45 and 50 ˚C), RPM (static, 50, 100, 120, 150 and 200), inoculum size (250, 500, 750 and 1000 µl.) carbon sources (glucose, xylose, mannose, galactose, lactose, maltose, arabinose, fructose, sucrose, sorbose and starch at 1% w/v), concentration of sucrose (10, 15, 20, 25 and 30 g/l) and organic nitrogen sources (malt extract, peptone, yeast extract, beef extract, potassium nitrate, ammonium molybidate ammonium oxalate, and ammonium sulfate).

3. Results And Discusion

1. Isolation, Determination of cell dry weight, exopolysaccharides of different bacterial isolates.

The interactions of the microbial community in the ocean to maintain such a biogeochemical cycle are well-known; metabolomics research by profiling a number of chemicals indicate that the organisms create to interact (14).

As a result, 10 bacterial isolates were obtained from the Red Sea. bacteria were cultured for three days to test their ability to synthesis of exopolysaccharides (EPSs). The bacterial cells were then separated and dried to a consistent weight at 80°C. Five volumes of ethanol were used to precipitate the crude (EPSs), which was then washed with acetone and dehydrated with ether. The cell dry weight, EPS, and productivity of 10 marine bacterial isolates were shown in Table 1. It was discovered that the maximum bacterial isolate (BS6) produced EPS 5.9 gm/L from 3.6 gm/L cell dry weight with Productivity 163.8 percent, whereas the lowest bacterial isolate (BS8) produced EPS 1.35 gm/L from 3.14 gm/L with Productivity 48.7%.

2. Determination of DPPH free radical scavenging activity

The chemical profile of EPS determines its biological potency, which is influenced by the fermentation conditions employed to grow EPS-producing bacteria (15). glycosidic linkage, Mono-sugar composition, chemical modulations, and other factors influence the conformation, extended side chain, and molecular weight of EPS derived from various sources .(١٦)

The existence of these elements contributes to EPS's ongoing health-promoting efforts. The EPS of marine bacteria has grown in importance in medicinal applications such as immunomodulation, anti-inflammatory, anti-tumor, and antioxidants (17).

As part of our search for new bioactive compounds from marine microorganisms and bacterial extracts with antioxidant potential, this study intends to isolate and biochemically evaluate EPS obtained from marine isolates. **Table (2)** demonstrated that the antioxidant activity of these EPSs varied, with the highest being BS6 (77.9%) at 120 minutes and the lowest being BS2 (28.3%) at 90 minutes, while four EPSs (BS 1, BS 3, BS 7, and BS 8) having no antioxidant scavenging activity. Table 3 shows the DPPH free radical scavenging activity (percent) for the marine bacterium BS 6 at various concentrations (1, 2, 3, and 4 mg) and times (15, 30, 45, and 60 minutes), revealing that BS6 has significant antioxedant activity (93.1 percent) after 120 minutes.

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

Isolate	CDW (gm/L)	EPS (gm/L)	Productivity (%)
BS 1	2.01	3.14	156.2
BS 2	1.68	3.56	211.9
BS 3	1.94	2.48	127.8
BS 4	1.72	3.92	227.9
BS 5	3.5	2.48	70.8
BS 6	3.6	5.90	163.8
BS 7	1.95	1.43	73.3
BS 8	3.14	1.53	48.7
BS 9	2.45	3.12	127.3
BS 10	2.98	3.66	122.8

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
BS 1	0	0	0	0
BS 2	10.6	15.7	21.1	28.3
BS 3	0	0	0	0
BS 4	12.4	18.9	26.5	33.3
BS 5	28.1	37.2	44.9	52.6
BS 6	35.8	51.0	68.4	77.9
BS 7	0	0	0	0
BS 8	0	0	0	0
BS 9	15.7	21.6	27.4	34.0
BS 10	31.5	39.4	46.5	61.7

Table (3) DPPH free radical scavenging activity (%) for BS 6

Concentration (mg/ml)	DPPH free radical scavenging activity (%)			
	15 min	30 min	45 min	60 min
30	10.3	25.7	48.4	63.2
60	32.5	65.1	70.3	81.3
90	40.1	69.5	78.9	89.1
150	43.5	75.6	86.2	93.1

3. Effect of some factors on the production of EPSs

Microorganisms' EPS output and composition are mostly determined by cultural circumstances such as temperature, pH, and medium composition (18). Sugars are primarily employed as a carbon source in the manufacturing of EPSs. However, there have been studies into the use of less expensive carbon sources, such as agro-industrial wastes or by-products (19).

The incubation temperature for fermentation medium is known to affect the growth of isolate (BS6), hence the maximum production of EPS (6.1 g/l) at cell dry weight (3.2 g/l) was seen at 40°C. Any variation in temperature reduces the formation of EPS **(Figure 1).** After three days of incubation, the maximum EPS productivity by (BS6), which produced 6.4 (gm/L) cell dry weight (3.4 g/l), was attained, and subsequently the EPS declined. These findings show that the relationship between EPS productivity and incubation duration varies per organism **(Figure 2).** The amounts of EPS produced by BS6 is influenced by the RPM **(Figure 3).**

At RPM 120, the maximum yield was 6.8 g/l, while the cell dry weight was 3.5 g/l. The size of the bacterial inoculum in the fermentation medium also has a significant impact on the synthesis of EPSs. **Figure (4)** At 750 l, the maximum EPS generation (7.0 g/l) was seen at cell dry weight (3.7 g/l). Changing the inoculum size in any way reduces the formation of EPSs. The level of EPSs produced by BS6 is controlled by pH. Thus, at pH 7, the maximum yield was 7.0 g/l and the cell dry weight was 3.8 g/l, indicating that either an excess of or a decrease in the pH of the medium resulted in reduced EPS synthesis **(Figure 5).**

Additionally, adding organic and inorganic nitrogen sources to the medium led in a significant increase in EPS synthesis. Peptone provided maximum EPS output (7.2 g/l) with cell dry weight (3.9 g/l) out of all the nitrogen sources examined. All of the other nitrogen sources produced varying amounts of EPSs **(Figure 6).** The least effective nitrogen source for the generation of EPSs (0.9 g/l) with cell dry weight (0.6 g/l) was potassium nitrate. When different carbon sources were introduced to the medium at a 1% concentration, the formation of EPSs increased. Sucrose produced the highest yield (7.2 g/l) at cell dry weight (3.9 g/l), while all other carbon sources produced a significant amount of EPS.

As a carbon source, starch was the least effective (0.3 g/l) (Figure 7). Figure (8), on the other hand, revealed that varying sucrose concentrations have an impact on the generation of EPS. Sucrose (20 g/l) yielded the highest EPS productivity (7.2 g/l), and cell dry weight was 3.9 g/l..

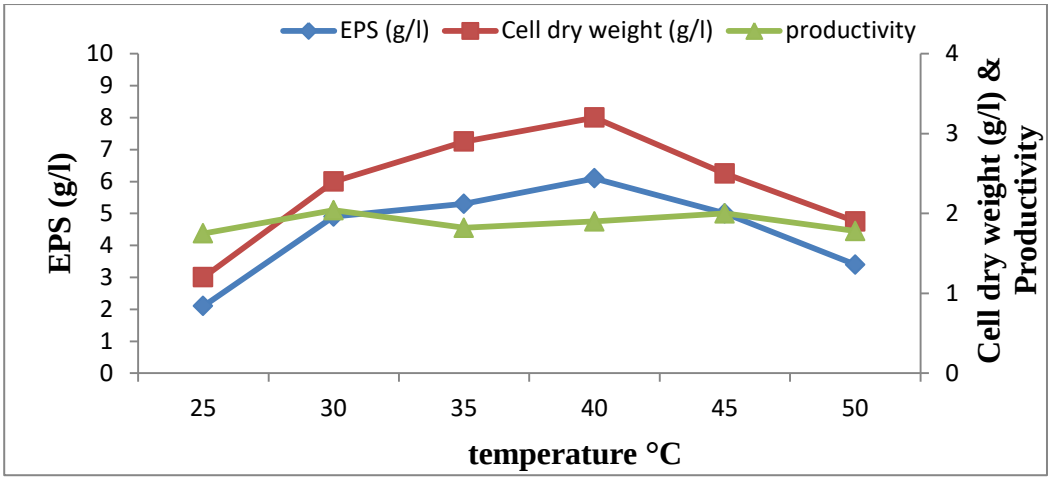


Fig. (1) effect of different temperature at production of BS 6

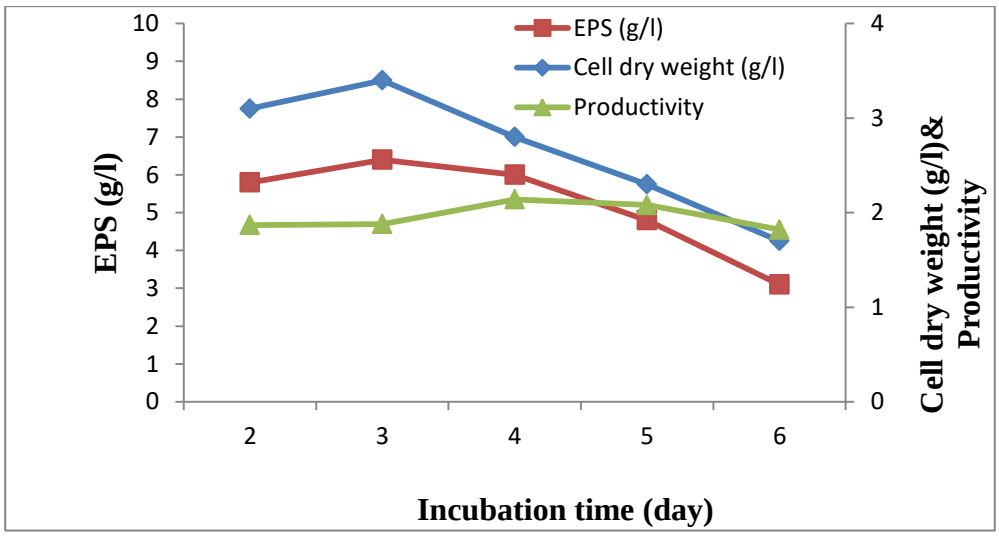


Fig. (2) effect of different incubation time at production of BS 6

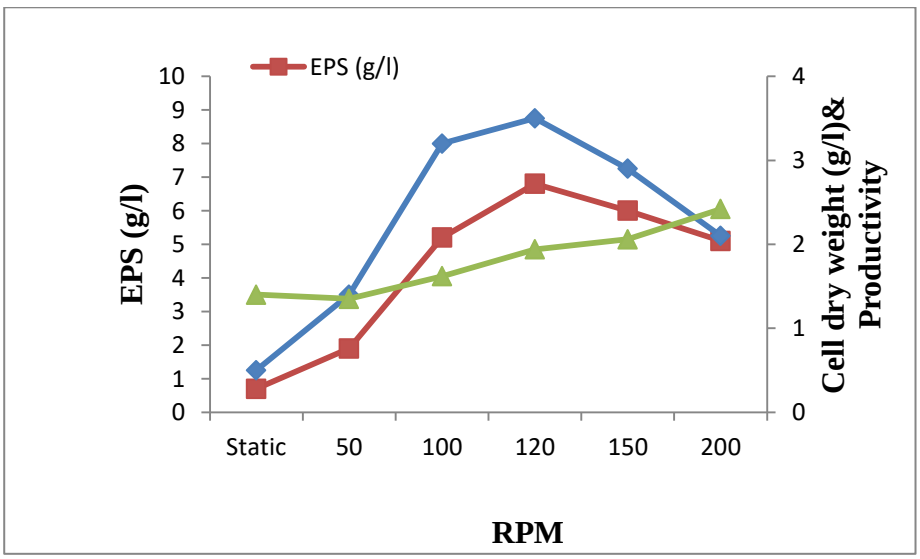


Fig. (3) effect of different RPM at production of BS 6

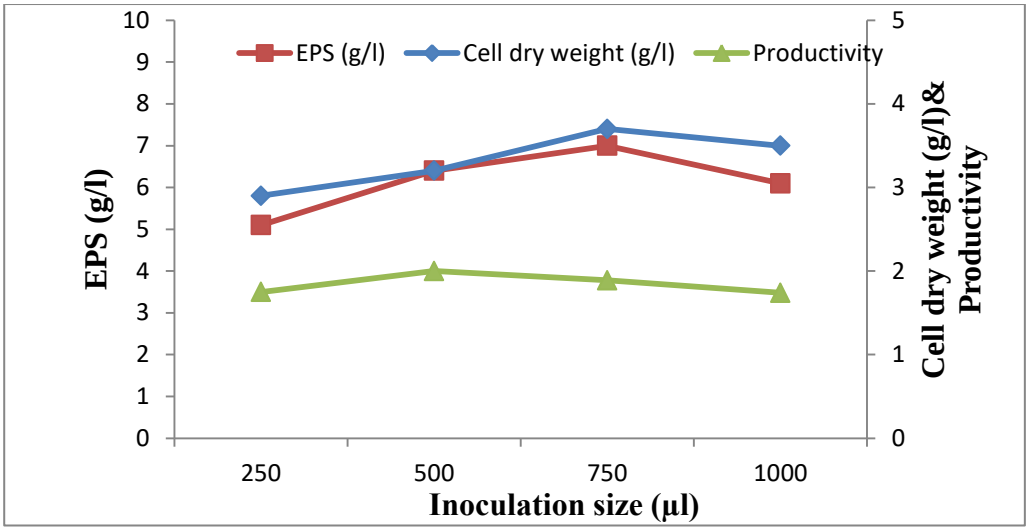


Fig. (4)effect of different Inoculation size at production of BS 6

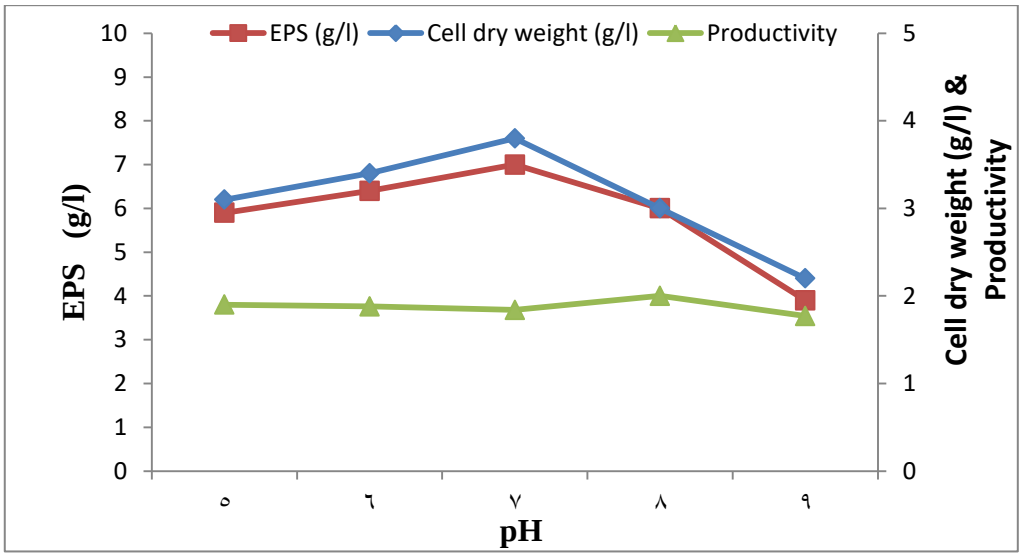


Fig. (5) effect of different pH at production of BS 6

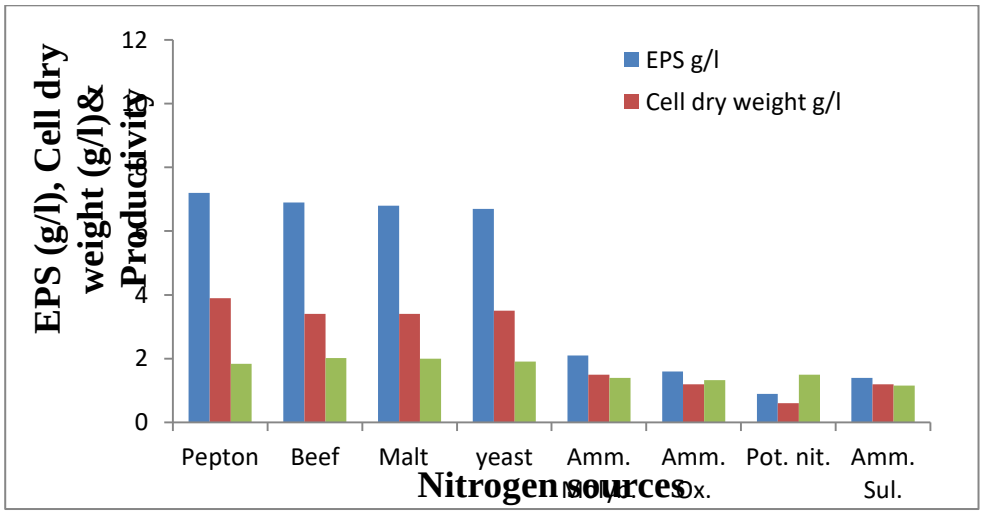
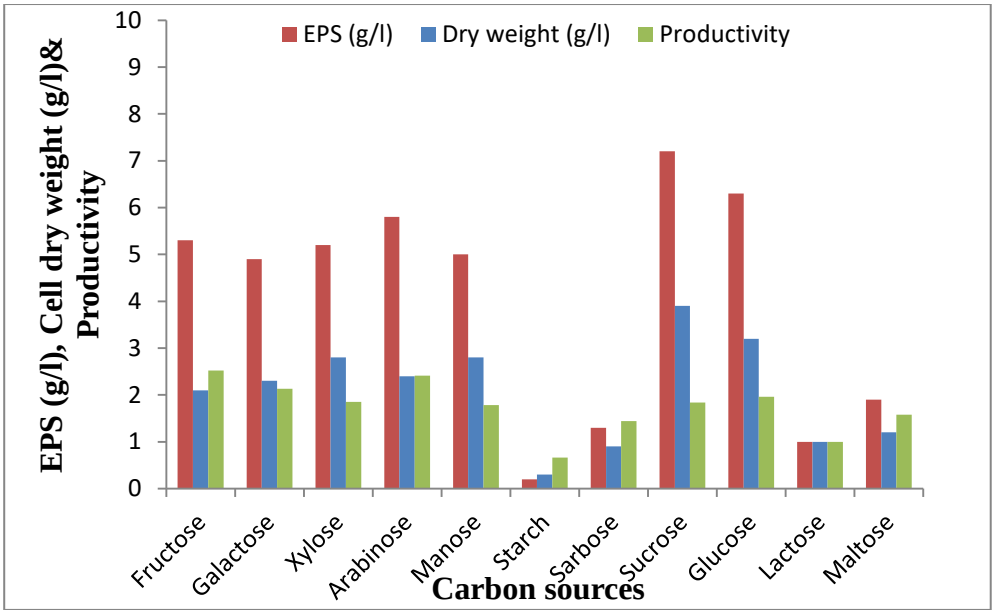
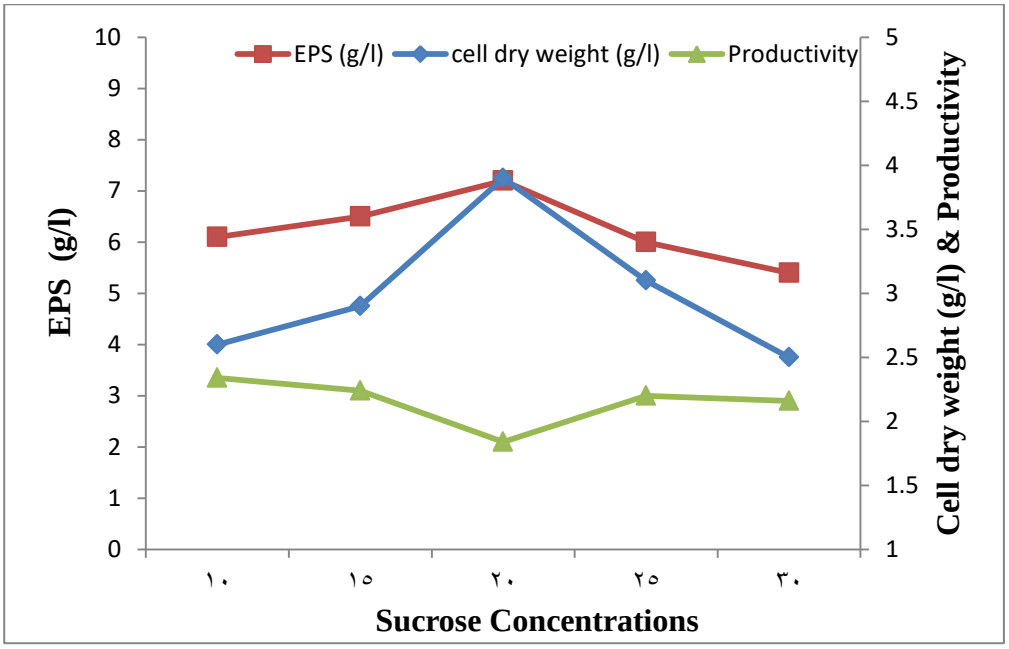


Fig. (6) effect of different nitrogen sources at production of BS 6



Fig(7) effect of different carbon sources at production of BS 6



Fig(8)effect of different sucroce concentration at production of BS 6

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