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Production and Optimization of Bioflocculant isolated from bacteria

Mervat.G.Hassan[1], M.E.El Awady [2], Sahar.S.Mohamed[2], H.W.Elbanna[1] and M.O.Abdel-Monem[1]
[1]Botany and Microbiology Department, Faculty of science, Benha University, 13518 Benha,Egypt
[2]Microbial Biotechnology Department, National Research Centre, El-Buhouth St. 33, Dokki-Cairo 12622, Egypt
E-mail: Hazembanna3@gmail.com.

ABSTRACT

Bacterial extracellular polymeric substances (EPS) contribute to the structure, surface charge, and settling properties of activated sludge by assisting in the development of bioflocs. From various soil sources, a total of twenty bacterial isolates were collected. The maximum bacterial isolate was (HS9), which produced (5.8 g/l) after being examined for exopolysaccharide (EPS) synthesis. As a result, EPSs were selected based on Bio-flocculant activity, while HS9 was the most active (86.95 %). The lowest was HS1, while the highest was HS2 (14.55 %). The synthesis and production of EPS are influenced by a variety of variables. As a result, by examining the effects of various factors on the production of EPSs by isolate (HS9), it was discovered that the maximum yield of EPS was (7.9 g/l) and cell dry was (4.1 g/l) when incubation temperature was 40 °C after three days, RPM 120, pH 7, peptone as a nitrogen source, and sucrose (20 g/l) as a carbon source.

Keywords: Exopolysaccharides, Optimization, Production, Bacteria, Bioflocculant.

INTRODUCTION

The term flocculation refers to the contact and adhesion process that allows dispersed particles to aggregate and form clusters, which then settle down to form the flock. In wastewater treatment, colloidal removal is a key priority since chemo physical techniques like flocculation are frequently used [8, 10].

There are three types of flocculating agents: (a) inorganic flocculants, such as aluminum sulphate; (b) organic synthetic flocculants, such as polyacrylamide derivatives; and (c) bio-flocculants, such as EPS [25]. Bio-flocculants are secondary metabolites produced by microorganisms (such as bacteria, fungus, and algae) during their growth. Proteins, Polysaccharides, nucleic acids, and lipids are commonly found in bio-flocculants [14].

The goal of the flocculation process is to clump microscopic particles together into well-defined flocks that settle down and can be readily removed, resulting in a clear solution (supernatant). Despite the several benefits that chemical or artificial flocculants provide, new research reveals a link between Alzheimer's disease aetiology and aluminium neurotoxicity [1, 16].

By increasing ionic strength and decreasing zeta potential, bioflocculants can destabilise colloidal particles, resulting in a reduction in the thickness of the diffuse portion of the electrical double layer. Alternatively, because they have specific macromolecular structures with a variety of functional groups (e.g.carboxyl and hydroxyl groups) that may interact with pollutants, they may adsorb counter ions to neutralise particle charge [15].

Bioflocculants have received a lot of scientific and biotechnological attention because of their biodegradability, benign end products, and application possibilities [2]. The recent demand for biopolymers for a variety of commercial applications has sparked interest in exopolysaccharide synthesis (EPS). They're usually long-chain, high-molecular-weight polymers made up of branching repeating units of sugars or sugar derivatives like fructose, glucose, galactose, and mannose that are created and released as the microorganism grows [7,22].

On the other hand, bio-flocculants, are still in the research stage and are hampered by issues such as uncertain production costs and non-uniform manufacturing procedures. Most bio-flocculant production now employs defined media as a fermentation feedstock, which is expensive, and alternatives such as complex media with high nutritional content are a good alternative [5].

On This way, this study was able to complete the screening and isolation of 16srRNA in this manner. Characterization of bio-flocculant-producing bacteria collected from various sources. The potent isolate was identified using morphological, biochemical, and physiological approaches, and was then used for a variety of applications, including sewage water treatment.

Material 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.) (1% tryptone, 0.5% yeast extract, 0.5% NaCl, 1.5% agar, pH 7) as described by [18]. Media were dissolved in 750 ml sea water completed to 1 L. by serial dilution method of [6 .[

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, FeSO4 0.01, peptone 5.0, agar15, g/l dissolved in 1 liter sea water: distal water (7.5:2.5), and the pH adjusted to 7 [4] 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 [21 .[

.٣ Determination of flocculant activity

With minor adjustments, flocculant activity was performed according to the method given by [9].In a test tube 4.5 ml of kaolin suspension (5,000 mgL−1) was added to 100 µL of the test bio-flocculant substances, vortexed for 30s and allowed to stand for 5 min at room temperature (37°C). At 550 nm, the upper phase's absorbance was measured (A). In the control experiment, 100 L of water was added to the suspension instead of bio-flocculant, with the rest of the parameters remaining the same as in the above experiment (B). (N=B-A/B X100) The flocculant activity (percent) was determined and calculated. The activity was calculated as the average of three measurements.

RESULTS AND DISCUSION

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

The increase in the number of free functional groups, which can act as bridges to carry many suspended particles together, caused the flocculating actions [3, 20]. A total of 20 bacterial isolates were obtained from the Red Sea at random locations. As a result, bacteria were cultured for three days to test their ability to synthesis exopolysaccharides (EPSs). The bacterial cells were then isolated 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 20 marine bacterial isolates were shown in Table 1. Productivity showed that strain HS9 had the highest flocculation rate (5.8 g/l) from (3.11 gm/L) cell dry weight (186.4%). while, the bacterial isolate with the lowest productivity was (HS11), which produced EPS (0.92) gm/L from (1.21) gm/L. (76.6 %).

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

Isolate	CDW (gm/L)	EPS (gm/L)	Productivity (%)
HS 1	1.96	2.69	137.2
HS 2	2.15	3.12	145.1
HS 3	1.38	2.01	145.6
HS 4	2.56	2.49	97.2
HS 5	2.96	2.61	88.1
HS 6	2.06	3.22	156.3
HS 7	2.55	1.69	66.2
HS 8	1.29	1.99	154.2
HS 9	3.11	5.8	186.4
HS 10	1.36	1.01	74.2
HS 11	1.21	0.92	76.6
HS 12	1.93	2.66	137.8
HS 13	1.69	3.14	185.7
HS 14	2.96	3.19	107.7
HS 15	3.05	4.55	149.1
HS 16	2.34	3.71	158.5
HS 17	2.22	2.59	116.6
HS 18	1.56	1.66	106.4
HS 19	1.88	2.09	111.1
HS 20	2.64	4.00	150.9

1. Determination of flocculant activity

For decades, flocculants have been widely employed in drinking water, wastewater treatment, fermentation processes, and food production [23]. Organic flocculant breakdown products are also classed as hazardous due to the release of monomers into the food chain, which can cause cancer [20, 24]. Because bio-flocculants are biodegradable in nature, they are increasingly used in wastewater treatment, downstream processing, and fermentation processes [17].

Table (2) shows the flocculant activity of these EPSs. The most active was HS9 (86.95%) at 60 minutes, while the least active was HS1 (14.55%). Ten EPSs (HS3, HS5, HS6, HS8, HS11, HS12, HS15, HS17, HS18, and HS19) had no flocculant activity. Table (3) shows the flocculant activity (percent) of the marine bacterial HS9 at various doses (0.1-1.0 mg/ml) and times (10, 20, 30, 40, 50, and 60 minutes), revealing that HS9 has a high flocculant activity (95.09 %) after 60 minutes.

Table (2) Flocculant activity (%) for marine bacterial EPS at different periods

Isolates no.	Flocculant activity (%)					
	10 min	20 min	30 min	40 min	50 min.	60 min
HS 1	10.36	11.33	12.06	12.99	13.64	14.55
HS 2	23.69	25.96	28.96	32.69	35.01	38.00
HS 3	0	0	0	0	0	0
HS 4	19.89	24.69	28.31	31.25	33.99	35.15
HS 5	0	0	0	0	0	0
HS 6	0	0	0	0	0	0
HS 7	31.66	35.66	39.88	45.21	49.11	56.22
HS 8	0	0	0	0	0	0
HS 9	48.81	53.70	67.47	71.32	78.31	86.95
HS 10	36.99	45.87	49.96	56.11	61.08	66.31
HS 11	0	0	0	0	0	0
HS 12	0	0	0	0	0	0
HS 13	11.02	15.63	18.21	21.09	25.31	29.67
HS 14	39.37	45.36	49.99	53.28	58.39	62.22
HS 15	0	0	0	0	0	0
HS 16	20.00	26.99	31.25	34.77	37.12	40.28
HS 17	0	0	0	0	0	0
HS 18	0	0	0	0	0	0
HS 19	0	0	0	0	0	0
HS 20	11.12	13.25	14.21	15.99	17.30	18.69

Table (3) Bioflocculant activity (%) for marine bacterial HS9

Concentration mg/ml	10 min	20 min	30 min	40 min	50 min.	60 min
0.1	30.59	39.85	45.22	53.45	59.04	64.09
0.2	35.65	40.26	47.95	56.28	63.16	69.15
0.3	38.29	44.33	52.15	57.64	66.14	74.64
0.4	42.14	48.81	58.36	67.19	75.36	80.38
0.5	48.81	53.70	67.47	71.32	78.31	86.95
0.6	55.56	60.18	69.84	78.85	85.44	89.11
0.7	63.89	68.44	74.53	81.19	87.18	92.82
0.8	66.21	72.93	79.77	88.66	91.70	94.13
1.0	71.09	78.19	86.61	90.07	93.96	95.09

2. Effect of some factors on the production of EPSs

Although significant progress has been made in the laboratory, large-scale bio-flocculant manufacturing has economic challenges due to the high cost of production and limited yield [11]. Carbon, Sulphur, Nitrogen, and phosphorus are the most important components for microbial medium in bio-flocculant production, and they account for the majority of the cost. Carbon sources such as fructose, glucose, and sucrose, as well as other classic media sources, are expensive components since they are professionally manufactured from precious and comparatively expensive resources [12].

The use of bio-flocculant yielding strains that are capable of degrading low-cost substrates, as well as growth conditions and substrate concentration optimization, are among the essential requirements for increasing bio-flocculant output from waste substrates [13, 19]. Industrial manufacturing of bio-flocculants has yet to be established due to the high cost of production. As a result, there is a demand for microorganisms with high bioflocculant production potential and low production costs [3].

The incubation temperature of fermentation medium is known to affect the growth of isolate (HS9), hence the maximum EPS production (6.0 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).** Following three days of incubation, the maximum EPS productivity was achieved by (HS9), which produced 6.3 gm/L cell dry weight (3.2 g/l), after which the EPS dropped. These findings show that the relationship between EPS productivity and incubation duration varies per organism **(Figure 2).**

EPS synthesize by HS9 is influenced by RPM (Figure 3). At RPM 120, the maximum yield was 6.4 g/l, while the cell dry weight was 3.3 g/l. The size of the bacterial inoculum in the fermentation medium then has a significant impact on the synthesis of EPSs. **(Figure 4)**

shows that at 750 l, the maximum EPS generation (6.8 g/l) was observed at cell dry weight (3.4 g/l). Changing the inoculum size in any way reduces the formation of EPSs. pH has an effect on the amounts of EPSs produced by HS9. Thus, at pH 7, the maximum yield was 7.0 g/l and the cell dry weight was 3.5 g/l, indicating that either an excess of or a decrease in the pH of the medium resulted in reduced EPS synthesis (Figure 5). While, effect of additional organic and inorganic nitrogen sources into medium resulted in a respectable increase in the EPS production. Peptone provided maximum EPS output (7.4 g/l) with cell dry weight (3.8 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 formation of EPSs (0.8 g/l) with cell dry weight (1.0 g/l) was ammonium sulphate. When different carbon sources were introduced to the medium at a 1% concentration, the formation of EPSs increased. Sucrose produced the highest yield (7.6 g/l) at cell dry weight (3.8 g/l), while all other carbon sources produced a significant amounts of EPS. As a carbon source, starch was the least effective (0.9 g/l) **(Figure 7).** While, **(Figure 8),** revealed that varying sucrose concentrations have an impact on the generation of EPS. Sucrose (20 g/l) yielded the highest EPS productivity (7.9 g/l), and cell dry weight was 4.1 g/l.

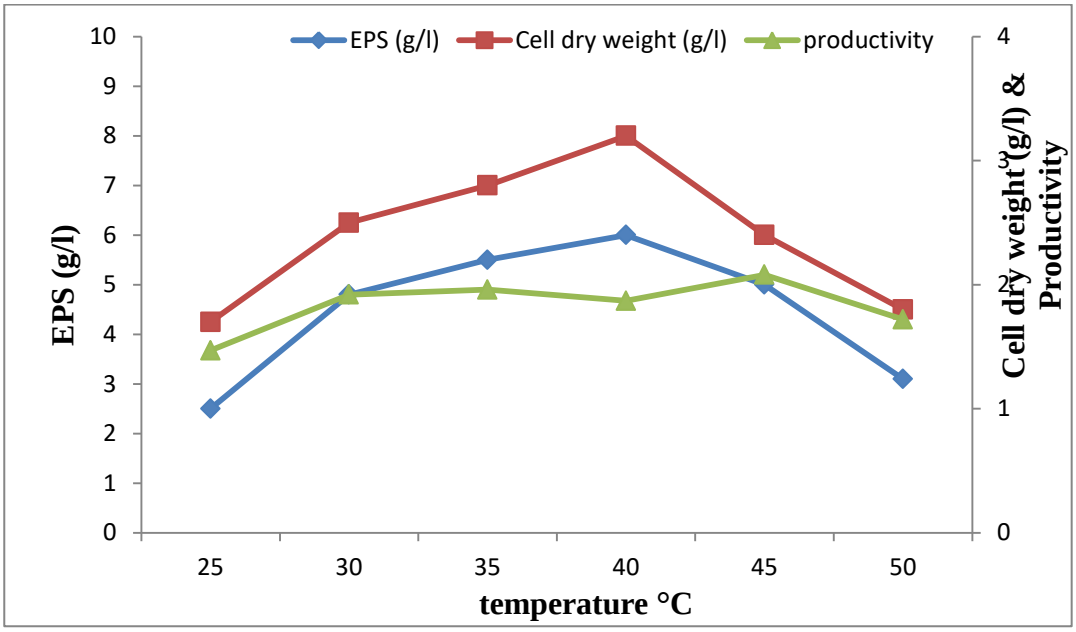

Fig. (1) Effect of different temperature at production of HS9

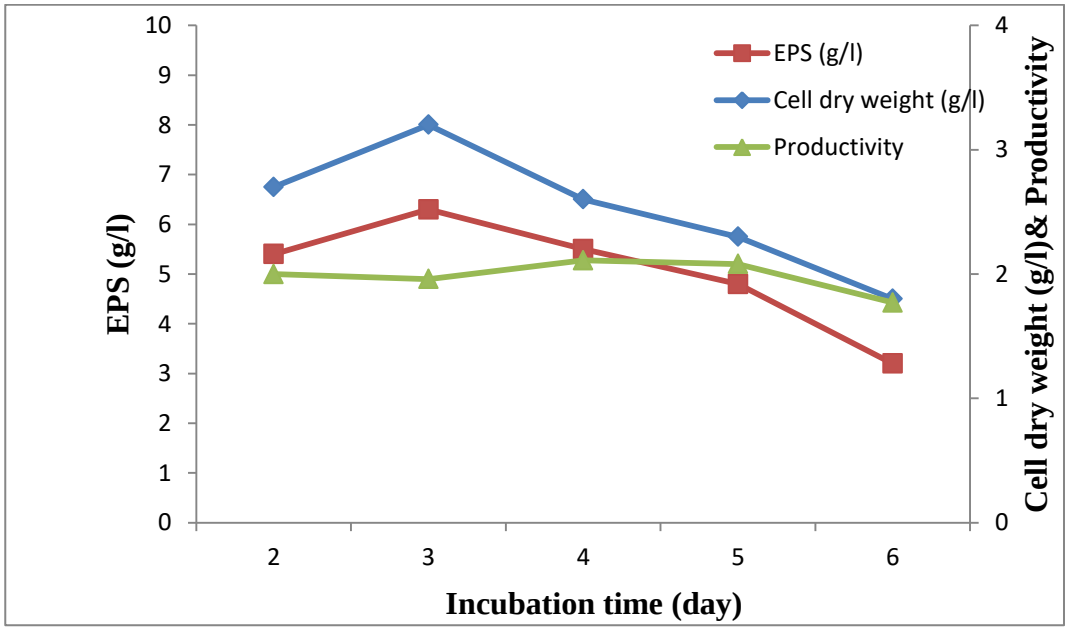

Fig. (2) Effect of different incubation time at production of HS9

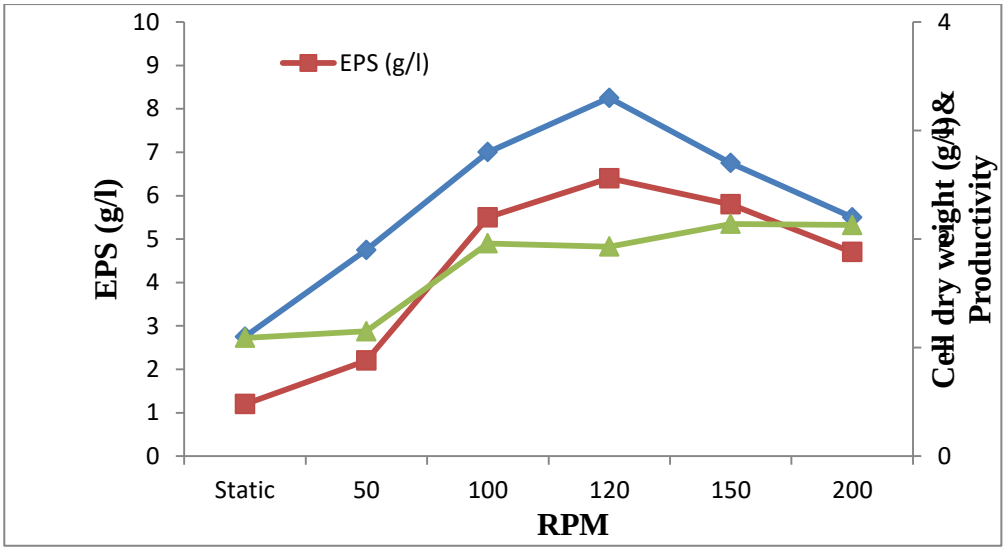

Fig (3) Effect of different RPM at production of HS9

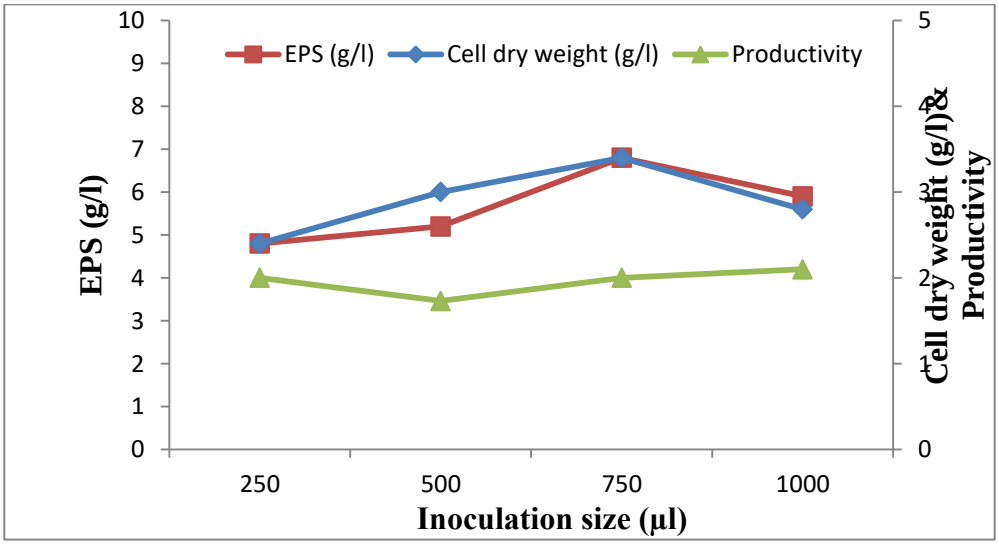

Fig. (4) Effect of different Inoculation size at production of HS9

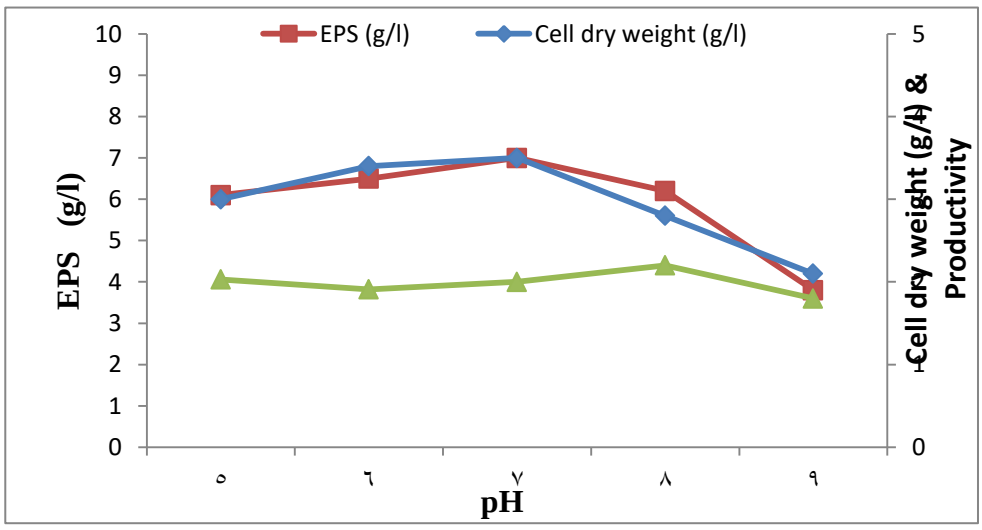

Fig. (5) Effect of different pH at production of HS9

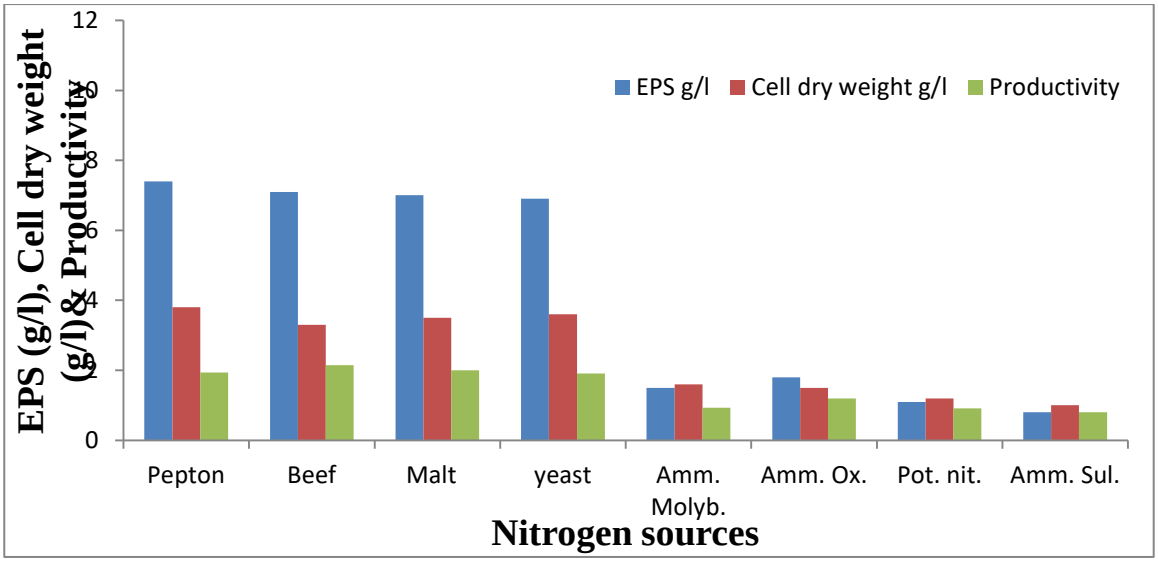

Fig. (6) Effect of different nitrogen sources at production of HS9

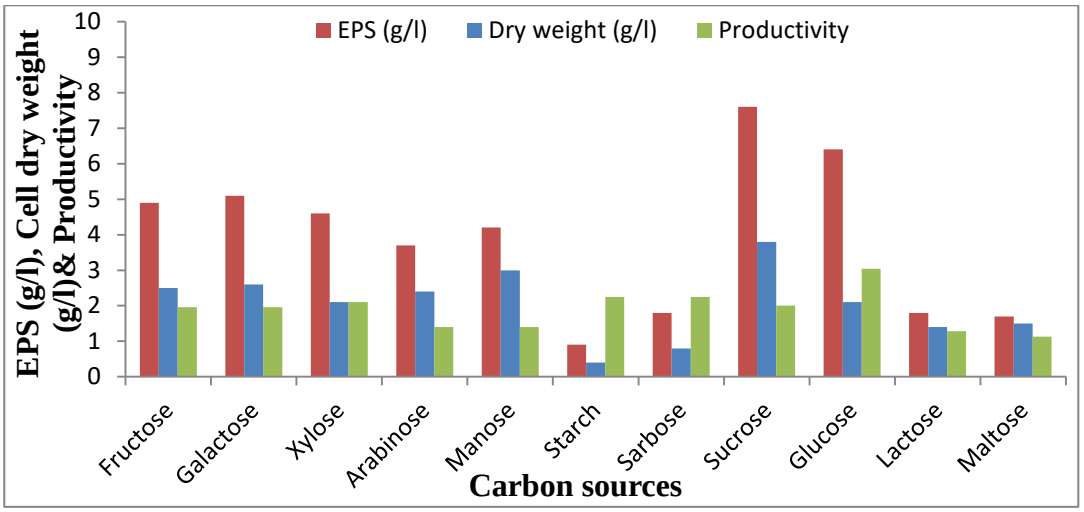

Fig. (7) Effect of different carbon sources at production of HS9

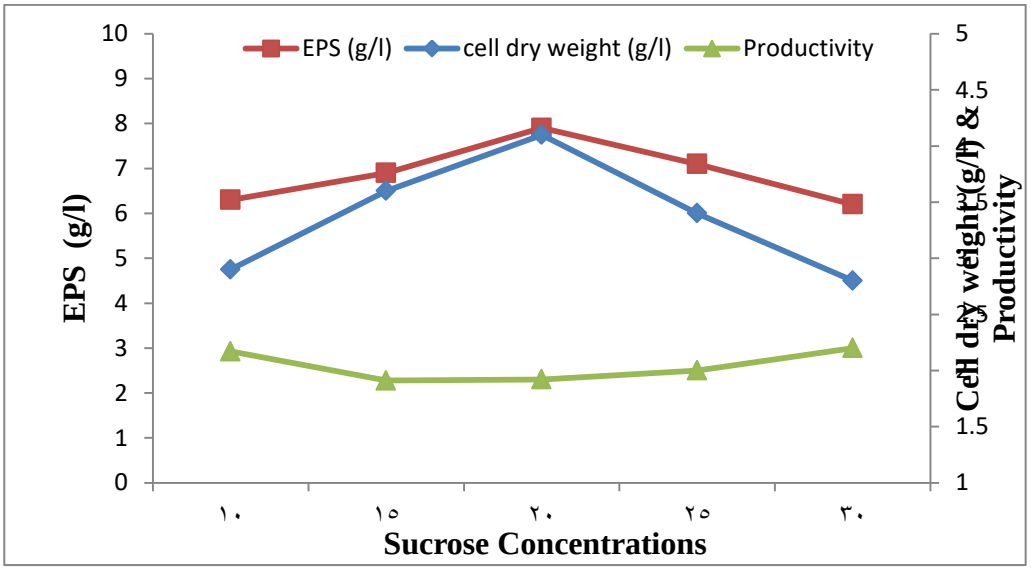

Fig. (8) Effect of different sucrose concentration at production of HS9

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