Organic manure (horses stool) affects the green alga Chlorella vulgaris Beijernick growth

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Abstract:

The green alga Chlorella vulgaris was inoculated with culture media of Chu-10 with or without different concentrations (0.5, 1, 1.5 and 2 gm l-1 dry weight) animal stools (manures) as an organic source of nutrient, for (12 days) at the laboratory conditions. The green alga utilized for growth, the phosphates and nitrates from organic sources rather than the phosphate and nitrate of the Chu 10 culture media.

The culture media contains of 2 gm l-1 organic manure gave more Chlorophyll a contents (6.72 µg l-1) after 12 days of the inoculation and more growth (71.5 X 103 cells.ml-1) was detected after 4 days of inoculation than that of other treatments., which it means that media had more ability to survive. Horse stool as an organic sources, found to be a good and cheap media for food nutrition.

Key world: Chlorophyll a, animal manures, nutrition.

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Introduction:

Organic sources (9) or waste products could affect the properties of the media and/or natural waters as a whole that caused serious problems (24). Such wastes may change the character of an aquatic environment. One of these effects is the fertilizing effect of some wastes that encourages the growth of plankton, algae and higher plants (29). This may due to the analysis of organic wastes by microorganism activities in to simple compounds such as nitrate, sulfate and phosphate. According to (11) excessive phosphate and nitrates may stimulate algal blooms to a large extent.(22) worked on the growth benthic fresh water algae on dairy manures and found that the untreated manures gave more growth than that of treated one.

The valuation of protein as a nutrition increased for human being and become more universal essential demand, due to highly increase in populations. Thus the scientists attracted their concern to look for this sector with a lot of attention (17; 20; 18).

Algal protein had been approved as the main alternative source to the human nutrition (30, 13, 2).(28) stated that the alga was found to be the most delicious food to the Rotifers,

the intermediate food chain. Espe and Lied (1999) prepared a cheapest highly qualified protein food to supply fish food, while(16) studied the food supply for chicks from red microalgae.

(19);(14) and(1) studied the effect of physical, chemical parameters of media on algal growth. While Simmons (1974) and 3, 5 6) worked on the phosphorus and nitrogen metabolism of the green algae Chlorella.

Earlier time, inorganic materials used by Beijernick (1890) as culture media for algal growth as a food. The scientific workers were started to use the organic animal stool as a food sources to the algae (32; 34; 22).

The aim of this study is to investigate the effect of manure (horses stool) added to the chlorella growth media on growth of Chlorella vulgaris under laboratory conditions.

Material and Methods: Isolation, purification and culturing of Chlorella vulgaris Beijernick:

Phytoplanktons were collected from Shatt Al-Arab River using the phytoplankton net (20 μ mesh). The collected sample was centrifuged (1000 r/m) for four minutes. The precipitate was diluted and cultured in solid culture media, incubated under sufficient illumination (White fluorescent tubes, 90 ц mol photon m-2 s-1) and temperature (25 °C). The process repeated several times. Isolation, purification was achieved according to (26). Pure culture of Chlorella vulgaris Beijernick was carried out. Finally, a mass culture of isolated pure green alga was prepared using (Chu No. 10) media as described by(1). The procedure was carried out under antiseptic conditions and fixed temperature and

illumination. The stock culture was ready for further experiments.

Preparation of growth media:

Animal manure (horses stool) was used in this experiment.

The manure was dried grinded and sieved, then autoclaved.

Different concentrations of the grinded manure powder (0.5, 1.0, 1.5 and 2gm l-1) were used, each set dissolved in 500ml of (Chu No. 10) media. All the prepared solutions were shake well, then sterilized by autoclave and neutralized to pH 7.2 in antiseptic conditios.

Each of the prepared media were inoculated by 5ml of Chlorella vulgaris Beijernick stock media culture and then incubated at constant temperature and illumination, for 12 days.

Estimation of phosphate and nitrate:

Absorption amount of phosphate- phosphorus (PO4-P) was estimated according to(22). Nitrate- nitrogen (NO3-N) according to(35) as described by(25).

Estimation of Chlorophyll a content:

The chlorophyll a was determined after extraction by 90% acetone solution as described by(25).

Estimation of algal cell account:

Two reading were taken, first at the starting time of inoculation and the second after 4 days. The account of the algal (number per one ml.) of culture media was estimated by means of haemocytometer method according to(21)

Results and Discussions:

Phosphate and Nitrate:

Generally the amount of phosphate and nitrate values of all

culture media were started to decrease. The maximum value of phosphate-phosphorus was recorded at Chlorella vulgaris media (Chu 10 + 2gm l-1) at the beginning of the experiment. While the minimum one was detected at the control (Chu 10 media only) after 10 days of the inoculation period. The absorption of phosphate- phosphorus values, at the control media was faster than that of other media (Table 1). The decrease of phosphate at the control media was coincided with increase of chlorophyll a contents, but there was decline of the chlorophyll a values after the fourth day of the inoculation or the experiment, which not happen with other treated media (Table 2). This means that the Chlorella vulgaris utilized the phosphate to grow from other sources rather than the chu10 media.

The same trend was applied with nitrate, where the maximum value of nitrate nitrogen at Chlorella vulgaris media (Chu 10 and 2gml-1) was recorded at the beginning of the experiment. And the minimum value of nitrate nitrogen at Chlorella vulgaris media (the control, Chu 10 only) was recorded after 12 days of the inoculation period.

(11) reported that the increasing phosphate and nitrates in growth media cause an increase in growth and that may stimulate algal blooms to a large extent.

(15) had infested a small pond with the filamentous alga Rhizoclonium sp. Over two months in the summer, they concluded that large amounts of N and P can be removed from a pond by harvesting filamentous algae could have slight effect on dissolved N and P levels in the water in relation to the amount removed.(27) did the same thing with hydrophytes and had the same results.

Table 1: Values of Phosphate-Phosphorus and Nitrate-nitrogen contents (mg l-1), at different Chlorella Vulgaris culture media.

Different concentrations of manures, as organic sources were used as well as the control (Chu 10).

Chu 10 +										
2 gm.Manure		1.5 gm.Manure		1 gm.Manure		0.5gm.Manure				
NO3-N	PO4-P	NO3-N	PO4-P	NO3-N	PO4-P	NO3-N	PO4-P	NO3-N	PO4-P	
10.31	0.79	9.95	0.71	9.10	0.68	8.20	0.66	8.35	0.65	Start(0)
9.56	0.71	9.42	0.70	9.02	0.70	7.82	0.56	7.25	0.53	2 days
8.81	0.63	8.41	0.61	8.16	0.52	7.45	0.44	6.73	0.31	4 days
7.69	0.45	7.75	0.58	7.82	0.44	6.82	0.37	5.94	0.21	6 days
6.10	0.44	7.55	0.43	5.78	0.34	5.85	0.33	5.22	0.18	8 days
4.80	0.31	5.18	0.45	5.15	0.22	4.93	0.221	2.11	0.05	10 days
3.09	0.20	4.99	0.22	4.61	0.22	4.28	0.23	1.86	0.07	12 days

Chlorophyll a contents:

Table 2 shows the amounts of chlorophyll a contents of Chlorella vulgaris at different culture media. The minimum amount of chlorophyll a was recorded at the control culture media (Chu 10 only) during second and tenth day of the inoculation, while the maximum amount of chlorophyll a was recorded at culture media of (Chu 10 + 2 gm. manure) at the last day (the twelfth) of the experiment. The biomass growth of Chlorella vulgaris in term of chlorophyll a contents, at the control culture media, was turned to decrease after 6 days of inoculation, while the two other media (Chu 10 + 0.5gm and 1gm manures) turned to decrease after 10 days of inoculation (Table 2). In this experiment the green alga Chlorella vulgaris gave more yield at culture media of (Chu 10 + 2 gm. manure)

than other treatments(22) worked on the green alga Scenedesmus and Blue green Gleocapsa and found that the later grow faster than the former and attributed that to the ability of the blue green algae to grow on the highly saline medium and prefer the high alkalinity compared with the green algae. Similar results have been confirmed by (9) when he compared between two chlorophytes (Chlorella vulgaris and Scenedesmus obliquus) and one cyanophyte (Spirulina platensis) and found that the cyanophyte had more ability of growth compared with the chlorophytes(6) found that the biomass of the Cyanophyte algae (Oscillatoria sp.) fed with manure was more than those fed with chemical source (NPK).

Table 2: Values of chlorophyll a contents (µg l-1), at different Chlorella Vulgaris culture media. Different concentrations of

manures, as organic sources were used as well as the control (Chu 10).

Chu 10 +					
2 gm Manure	1.5 gm Manure	1 gm Manure	0.5gm Manure		
4.21	4.45	5.12	4.23	4.10	Start (0)
5.56	5.12	4.89	4.15	4.01	2 days
6.50	5.75	5.76	4.98	4.82	4 days
6.53	5.96	5.93	5.21	4.61	6 days
6.53	5.96	5.95	4.95	4.38	8 days
6.55	5.78	5.78	4.72	4.04	10 days
6.59	4.18	4.81	4.19	2.25	12 days

Algal cell account:

The growth in term of individual cell account is reported in Table 3. The green alga Chlorella vulgaris that was fed with Chu 10 +2gml-1 manure gave more growth than that of other cultures during the fourth day of inoculation, while the control culture media less growth than that of the others.

From the figures mentioned in Table 3, conclusion could be

arises that addition of the animal manure (horses stool source) as nutrient source with algal growth media could improve algae growth, and this is agreed with (33) and (2).

Table 3: Cell number values of Chlorella vulgaris species (X1000 unite ml-1), at different concentrations of manures culture media, as organic sources were used as well as the control (Chu 10 without manure).

Chu 10 +					
2 gm Manure	1.5 gm Manure	1 gm Manure	0.5gm Manure		
45.2	45.8	46.3	45.1	44.5	Start (0) Unit/ml. X1000
73.5	61.7	62.1	56.2	50.1	4 days Unit/ml. X1000

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تأثير إستخدام الفضلت العضوية (براز الحصان)

على نمو الطحلب الخضر Chlorella vulgaris Beijernick

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الخلاصة:

غذي الطحلب الاخضر Chlorella vulgaris بوسط زرعي يتكون من وسط (CHU 10)مضافاً اليه عدة تراكيز(1.50, 1.00, 0.5و2.00غم وزن جاف باللتر) من فضلات الخيول المجففة والمعقمة ولمدة 12 يوماً في ظروف مختبرية. وقد لوحظ ان الطحلب الاخضر يعتمد على المادة العضوية (فضلات الحيوان) في امتصاص الفسفور والنتروجين واستهلاكها في نموه وتكاثره أكثر مما يعتمد على الوسط الزرعي(Chu10).

نمو الطحلب بقياس كمية الكلوروفيل (المادة الخضراء) يكون عالي جداً بالوسط الذي يحتوي الوسط الزراعي (Chu10) مضافاً اليه المادة العضوية (فضلات الحيوان 2.00 غرام مادة جافة للتر الواحد) حيث يستمر نموه الى نهاية التجربة قيس نمو الطحلب بحساب عدد الخليا الطحلبية في بداية التجربة وبعد اربعة ايام من الزراعة فوجد ان نفس الوسط الزرعي (Chu 10) مضافا ال يه ال مادة العضوية (فضلات الحيوان 2.00 غرام مادة جافة للتر الواحد) يحتوي على اكثر عددا من بقية الوساط المزروعة خلل التجربة.