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Microbial load in mass cultures of green algae *Scenedesmus acutus* and its processed powder

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Abstract. Microbial contamination in cultures of the alga, *Scenedesmus acutus* raised in outdoor open tanks and also in the processed powder of the alga was monitored; The total bacterial population increased with time during the growth period of six days. When a combination of molasses and carbondioxide was employed as carbon source for this alga, the bacterial load increased to 10 colony forming units/ml. Yeast, molds and also coliforms were quantitated. Drum-drying the algae drastically reduced the bacterial load and storing the algal powder for a period of over 3 months did not increase the bacterial load. Pathogens like *Salmonella* and *Staphylococcus* were not detectable either in the open cultures or in the drum-dried algal powder. Although there are not set standards available in literature on the permissible level of the microbial contamination in algal biomass for use in foods, the microbial load appears to be within the limits of permissible levels stipulated by Indian Standard Institution standards for baby foods.

Keywords. Green alga; *Scenedesmus acutus*; microbial load; bacterial contaminants; processed powder.

Introduction

Algae are receiving wide attention as a source of biomass protein (BMP) for use in animal feeds and foods (Mituya *et al*., 1953; Mateles and Tennenbaum, 1968; Clement *et al*., 1969; Dabah, 1970; Soeder and Pabst, 1975; Becker and Venkataraman 1978; Becker *et al*., 1976). The technology of algal cultivation and processing is now known for a few algae like *Chlorella*, *Scenedesmus*, *Coelastrum* and *Spirulina*. Clean water production of the green alga *Scenedesmus acutus* has been standardized at this Institute (Venkataraman *et al*., 1977a; Becker and Venkataraman, 1978). In this procedure, the alga is mass cultured in open cultivation basins and sterile conditions cannot be maintained. Under these conditions, the culture may be contaminated with heterotrophic bacteria even when it is grown autotrophically with simple inorganic media (Mituya *et al*., 1953; Leone. 1963; Blasco, 1965; Vela and Guerra, 1966).

The possible health hazards of such contamination in mass algal cultures for animal feed and food cannot be overlooked. While extensive investigations have been carried out on the nutritional and toxicological safety of the algal biomass

Abbreviations used: BMP, biomass protein; efu, colony forming units.

(Soeder and Pabst, 1975; Becker *et al*., 1976; Subbulakshmi *et al*., 1976; Venkataraman *et al*., 1977b and 1980), very little is known about the microbial contaminants. No reports are available on the bacterial load either in cultures or in processed material.

The common occurrence of different species of bacteria in algal cultures and in processed products raises the question of their significance in regard to public health. Microbiological guidelines or standards for processed algal products are needed to ensure the quality of the products and to protect consumers from hazards.

The present study is an effort to determine the bacterial load and to quantitate and identify the organisms in algal cultures and processed algal powder. The study was also intended to focus attention on the bacteriological aspects of algal biomass production.

Materials and methods

Cultivation of algae

Scenenedesmus acutus 273-3a was used in this study. Stock cultures were maintained on agar slants. Inoculum for outdoor culturing was prepared from slants in flourescent light illuminated thermostats with a light intensity of 5-7 K.lux, Air was passed into the culture tubes at a rate of 300-500 ml/min. From the culture tubes, the algae were transferred to glass carboys to build up enough inoculum for mass culturing outdoors. The detailed procedure has been published elsewhere (Venkataraman *et al*., 1977a; Becker and Venkataraman, 1978). The algal cultures both indoors and outdoors were grown under non-aseptic conditions.

The algal cultures in outdoor tanks were concentrated by a centrifuge separator and dried on a drier (electrical or steam heated) or sundried on plastic sheets.

Growth Measurements

Algal growth and consequently the biomass produced are expressed as mg of algae produced/litre culture. This was computed by measuring absorbance of the cultures at 560 nm and converting the same into algal biomass with the help of a Standard graph. The normal growth period of *Scenedesmus* cultures when it is ready for harvest is about 6 days, and hence all measurements were taken for this duration alone.

Storage

The dried algal biomass was stored in polyethylene-lined aluminium bags and kept for a period of three months for storage studies.

Sample preparation

For all the assays that are reported here, 10 ml of algal culture or 10 g processed algal powder were used. The material was mixed with 90 ml of 0.1% sterile peptone water. This was shaken for 10 min and suitable dilutions were made for the various assays.

Determination of viable count:

Bacteria: Standard methods were followed to determine the plate counts using nutrient agar media (Harringen and MacCance, 1976). Counts were expressed as colony forming units (cfu)/ml of culture or per *g* of algal powder.

Yeast and molds: These were assayed on acidified potato-dextrose medium. The plates inoculated with the samples were incubated at 25°C for 5 days followed by counting.

Coliforms: Coliform counts are expressed as most probable number using MacConkey broth (Harrigen and MacCance, 1976). Differential tests were used in the identification of the coliforms as *Escherichia coli, Enterobacter aerogens* and intermediate species (Speck, 1976).

Standard methods were used for determining the coagulase positive *Staphylococcus aureus* (Thatcher and Clerk, 1968). Suspected colonies were tested for coagulase activity. Analysis for Salmonella was carried out using the method of Thatcher and Clerk (1968).

Biochemical tests

The bacterial forms that were quantitated on the total plate counts were identified based on colony characteristics and biochemical tests as detailed in Bergey's manual (Breed *et al*., 1957).

Results

Algal growth

As can be seen from table 1, the growth of alga with the addition of molasses alone was lower than with molasses and CO_2. The level of molasses used in the study appears to be optimal to prevent serious contamination by bacteria. This is evidenced by the absence of sugars in the culture medium when tested the following day after the addition of molasses.

Bacterial load

The bacterial load as a function of the age of cultures increased both in indoor and mass outdoor cultures. The increase was much less in indoor cultures during inoculum development as compared to the open basin algal cultures exposed completely to the environment. The bacterial load of cultures monitored at 0, 3 and 6 days of growth are summarized in table 1. The contamination is possibly from water and also from air. The excretion of certain organic substances by the algal cells into the media which may support bacterial growth has been reported in algal cultures that are grown in simple inorganic media (Vela and Guerra, 1966).

In indoor cultures, coliform group of organisms was not detected. In outdoor cultures, bacterial load increased from 0.3 to 45.0×10^5/ml within 6 days. However, there are some reports that the bacterial levels remain stable without significant increase in *Chlorella* cultures (Mituya *et al*., 1953; Leone, 1963). Details of cultivation methods and the age of cultures at which the bacterial measurements were taken are not available for the above and hence this cannot be explained.

Table 1. Microbial load in *Scenedesmus acutus* cultures raised indoors and outdoors.

Conditions of cultures	Age of culture (days)	mg dry algae/ L	Total colony forming units/ml $\times 10^5$	Yeast & molds (colonies/ ml$\times 10^2$)	Coliform group (Most probable number/ml)
Indoor culture	Initial	60	–	–	Nil
(Raised in	3	140	1.2	1.0	Nil
thermostats)			(1-5)[a]	(0.5-1.5)	
	6	280	15	4-5	Nil
			(8-25)	(3-9)	
Mass outdoor	Initial	80	0.3	1.0	Nil
cultures			(0.2-4.0)	(0.3-2.0)	
(Autotrophic	3	200	18	5.0	29
culture			(8-25)	(3-8)	(11-75)
30L CO_2/h)	6	320	30	15	280
			(25-60)	(7-25)	(210-460)
Heterotrophic	Initial	80	0.3	1.0	Nil
culture (100 mg			(0.2-4.0)	(0.3-2.0)	
of sugarcane	3	170	12	1.8	120
molasses/L)			(5-20)	(1.0-6)	(75-210)
	6	260	45	5.0	180
			(25-80)	(4-10)	(120-240)
Mixotrophic	Initial	80	0.3	1.5	Nil
culture (30 L			(0.2-4)	(0.3-2)	
CO_2/h and	3	290	30	10	300
100 mg sugar cane			(20-80)	(3-25)	(240-460)
molasses/L)	6	480	400	30	900
			(200-500)	(20-60)	(460-1100)

[a] Values in parenthesis indicate range

–Not detectable.

The values represent mean of five independent observations.

The coliform group of organisms was found only in a few batches. This type of contamination cannot be avoided under the pattern of cultivation used here. By suitable processing methods, they can be eliminated in the final product. Yeast and mold counts also increased in algal cultures during the growth period of six days.

Effect of organic carbon source

Sugarcane molasses is being considered as an alternative or supplementary carbon source to CO_2, which is rather expensive. Addition of sugarcane molasses even at a low level enhances the bacterial load in the cultures and this may be attributed to

the presence of simple sugars in the medium. It is also possible that bacteria may oxidize the small amount of molasses rapidly and liberate CO_2 into the medium, thus stimulating algal growth.

Mixotrophic cultures have much higher bacterial load (4.0×10^7 cfu/6 days growth) compared to heterotrophic cultures. This needs serious consideration as the algal growth and consequently the biomass productivity is higher in mixotrophic cultures.

Pathogenic forms

The pathogenic forms like *Salmonella* and *Staphylococcus* were not found in the cultures at any stage of growth of algal cells.

The major contaminating organisms were identifed by biochemical tests of one hundred randomly picked colonies. The results are listed in table 2. *Micrococcus* species were more common than those of Bacillus. Coliforms were much less frequent compared to other bacterial species. The various organisms of the coliform group identified are listed in table 3.

Table 2. Bacterial forms isolated from outdoor open Scenedesmus acutus cultures[a]

Organism	No. of isolates (%)
Micrococcus ureae	10
Micrococcus roseus	10
Micrococcus flavus	5
Micrococcus cryophilus	5
Micrococcus freundenreichii	8
Micrococcus varians	6
Micrococcus conglomeratus	10
Micrococcus caseolyticus	8
Bacillus subtilis	5
Bacillus coagulans	8
Bacillus macerans	3
Bacillus megaterium	2
Bacillus lentus	4
Bacillus brevis	2
Bacillus alvei	1
Bacillus circulans	4
Bacillus licheniformis	3
Unidentified forms	6

[a] Per cent occurrence of each bacterial species out of 100 randomly isolated colonies.

Table 3. Identification of coliform from mass outdoor cultures of *Scenedesmus acutus*[a]

Total number of isolates	Species of bacteria	Type I[b]	Type II[b]
100	*Escherichia coli*	20	5
	Intermediate	15	20
	Aerobacter	17	23

[a] Per cent distribution of the various types among one hundred randomly selected coliform colonies.

[b] Type I and II classified based on indole; methyl red; VP (Voges-Proskauer) and Citrate tests.

Effect of different methods of processing:

Sundrying, and drum-drying on electrical or steam heated driers have been used to prepare algal powder form the slurry. The microbial load in the dried powder is much lower than in cultures as would be expected (table4). Drum-drying sterilizes the algal cells at least partially in spite of the short detention time (8-10 s). Drum-drying on electric dryer appears to reduce the microbial load and yeasts and molds more effectively than a steam-heated drier. This is probably due to lesser drum temperature being attained in the steam-heated drier.

Table 4. Microbial load in dried algal powder processed under different conditions[a]

Types of drying	Carbon source used in culture	Colonies forming units (c.f.u)/g ($\times 10^4$)	Yeasts and molds colonies/g ($\times 10^2$)
Electrically-heated drum drier	CO_2	2.0 (0.8-3.0)	1.0 (0.5-2.0)
Steam-heated drum drier	CO_2	3.0 (2.0-6.0)	6.0 (5.0-8.0)
Steam-heated drum drier	CO_2+ molasses	10.0 (5.0-15.0)	25.0 (10.0-30.0)
Sun dried	CO_2	120 (100-180)	140 (100-210)

[a] Products were free from *Staphylococcus*, *Coliform* group and *Salmonella*.

Values represent mean of five independent observations. Figures in parenthesis indicate the range of values.

When the cultures are grown mixotrophically with CO_2 and molasses, the microbial load is slightly higher than those grown with CO_2 alone. This may be due to the presence of spore-forming bacteria and also due to higher microbial load in the algal culture itself (table 1). The sun-dried algae had much higher microbial load than drum-dried material. No *Salmonella* and *Staphylococcus* were detected in the differently processed powders. Coliforms were totally absent in dried algal powder even though they were present in algal culture; possibly, they are destroyed during drying. The types of bacterial forms isolated from drum-dried algal material are listed in table 5. This differs significantly from the algal cultures in the total absence of the various species of *Micrococcus*.

Table 5. Bacterial forms isolated from drum-dried *Scenedesmus acutus* powder[a].

Organism	No. of isolates (%)
Bacillus subtilis	20
Bacillus coagulans	25
Bacillus macerans	10
Bacillus alvei	5
Bacillus brevis	5
Bacillus lentus	10
Bacillus circulans	10
Unclassified	15

[a] Per cent occurrence of each bacterial species out of hundred randomly isolated colonies.

Storage studies

Data on storage studies conducted for a 3-month period using drum-dried algal powder are given in table 6. There was no increase in microbial load. This may be due to low moisture content (6-8%) which is not suitable for bacterial growth.

Table 6. Microbial counts on stored dried algal powder.[a]

Age of the powder (days)	Total plate counts Colonies/g $\times 10^4$	Yeast and moulds Colonies/g
Initial	9.0	600
15	7.0	550
30	7.0	550
45	6.5	500
60	6.0	450
90	5.5	400

[a] Values represent mean of three independent observations. Temperature 28 ±2°C.

Discussion

Microbial monitoring of algal culture and algal powder is necessary, since it is likely to affect the quality and safety of the final product. There are no internationally accepted safety limits that are available to draw a definite conclusion as to whether *Scenedesmus acutus* grown on a pilot plant scale at this Institute are within permissible limits.

However, the *Scenedesmus* grown in clean water and the drum-dried powder have microbial loads within probable safety limits, when compared to Indian Standards Institute (ISI) guidelines for baby foods which is 50,000 colonies/g. The absence of pathogenic forms like *Salmonella* and *Staphylococcus* makes the algal powder safe for utilization. Drum-drying of *Scenedesmus* is an absolute necessity in order to rupture the undigestible cellulosic cell-wall for protein digestibility

This evidently gives an effective sterilizing effect, reducing the microbial load. Sun-drying is not a suitable method for processing *Scenedesmus* for the same reason and this had been confirmed by earlier studies (Subbulakshmi *et al*., 1976). Scenedesmus cultures are also contaminated by other organisms like protozoans, other algal species and water insects and this aspect is not detailed here. However, it may be mentioned that contamination of *Scenedesmus* by these is very limited and does not endanger either the culture or safety in its use as food.

The present world-wide emphasis has been more on technological and nutritional aspects connected with algal biomass production. Though the possibility of using algae as supplementary food protein has become less promising its use in feed is a distinct possibility and the bacterial load is an important criterion for safety of use. This should also receive more attention from the researchers in the field.

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