Cultivation of *Spirulina* in sewage for poultry feed[1,2]

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Summary. A method for cultivating *Spirulina platensis* in domestic raw sewage, coupled with pisciculture and water reclamation in an integrated recycling system, has been standardized. The alga is grown in an indigenously designed open-air pilot production unit consisting of 4 concrete basins with a total surface area of 450 m^2. The harvesting and processing methods are based on simple filtration and sun drying. Extensive bench and field experiments have made it possible to produce pure blooms of African *Spirulina* in sewage, using sodium bicarbonate and nitrate, and employing a fertilizing schedule which replenishes nitrogen withdrawn from the medium by the alga. Although urea and several ammoniacal nitrogen sources have been tried, the best source of protein-inducing nitrogen for mass cultivation of *Spirulina* appears to be nitric nitrogen.

Maximum long-term yields of 7.3-9.5 g $m^{-2}day^{-1}$ have been attained during the summer months, but they decrease to about 5 g $m^{-2}day^{-1}$ during the winter.

The chemical analysis of sewage-grown *Spirulina* revealed a high content of protein (50-55%) with a well-balanced amino acid spectrum, moderate quantities of carbohydrates and lipids, considerable amounts of pigments and low amounts of nucleic acids, nitrite and nitrosamines.

Microbiological analysis of the product has shown that the flora consists mainly of gram-positive, spore-forming bacilli and faecal streptococci; occasionally very low numbers of coliforms are detected. Pathogens, such as *Salmonella, Shigella* and *Vibrio* are absent. The material has also been found to be free from pathogenic amoebae, enteroviruses and worm cysts.

The results of the chick-feeding tests demonstrate that *Spirulina* protein can totally replace groundnut cake, the chief source of vegetable protein supplement in starter poultry feeds. In laying hens the alga shows better pigmentation effect on egg yolks than conventional oxycarotenoid sources and can be used as the sole pigmenter to obtain adequately colored yolks for table use or for the bakery industry.

The diluted effluent is fed at various flow rates into a 260 m^2 fish pond in which attempts are being made to rear valuable carp.

The water from the fish pond shows moderate amounts of dissolved solids and low values of sodium adsorption ratio and can, therefore, be safely utilized for irrigation purposes.

Spirulina has a great possibility for practical use in poultry feed and may be of interest especially for tropical countries of the Third World where new sources of feed may be important.

Introduction. The possibilities of mass culturing of microalgae have been actively investigated for over thirty years[4,17]. Among the blue-green algae, species of *Spirulina* are receiving greater attention as a food source. *Spirulina* grows prodigiously in brackish soda lakes in Central Africa and Mexico, and traditionally has been consumed by the tribal people in these regions[15,19]. The alga has an unusually high protein content (up to 70%), considerable amounts of vitamins, pigments and minerals, moderate quantities of lipids and carbohydrates, and a low content of nucleic acids[9,23].

Although certain species of bloom-forming cyanophytes are known to produce toxic strains which cause poisoning in animals[6], *Spirulina* has a safe history of consumption by humans. The alga does not appear to build up toxic or antinutritional substances and has no deleterious effects in animals or humans[7,10]. Another advantage of *Spirulina* is the large size of its filaments which allow harvesting by simple filtration.

Considering the nutritive value of *Spirulina* and the obvious ease of its harvesting, the alga has been the subject of a number of mass culture studies in several countries[8,11,12,16,18,21,22,25,26]. At present, an industrial pilot plant in Mexico produces one ton of *S. maxima* biomass per day[13].

We have developed a biotechnology for the production of *S. platensis* in sewage with view to utilizing domestic waste for algal biomass production for poultry feed, fish-culture and water for irrigation in an integrated pilot plant system, and also to help minimize environmental hazards and maximize ecological benefits[24].

Growth potential of Spirulina on sewage. As a first step in preparing the culture for the large-scale experiments the alga was grown indoors under controlled conditions, using a basal medium containing the following ingredients per l distilled water: 15.0 g $NaHCO_3$, 0.5 g K_2HPO_4, 2.5 g $NaNO_3$, 1.0 g K_2SO_4,

Table 1. Effect of levels of bicarbonate and nitrate on the culture density of *Spirulina* (g m^{-2} dry wt) in cultivation ponds after 12 days

HCO_3 (%)	No_3 (%)				
	0.25	0.1	0.05	0.01	0.005
1.8	120	116	68	46	16
1.0	112	108	60	42	12
0.5	97	67	52	33	7
0.25	93	56	41	25	4
0.1	71	49	37	20	4

1.0 g NaCl, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.04 g $CaCl_2 \cdot 2H_2O$, 0.01 g $FeSO_4 \cdot 7H_2O$, 0.08 g EDTA. The basal medium was enriched with 1 ml l^{-1} of an oligo-element solution containing: 2.86 g H_3BO_3, 1.81 g $MnCl_2 \cdot 4H_2O$, 0.22 g $ZnSO_4 \cdot 7H_2O$, 0.08 g $CuSO_{4\,2}O$ and 0.01 g MoO_3 per l distilled water.

Experiments on the potential of growth of *S. platensis* under outdoor conditions were conducted in 10 m^2 basins operated at a depth of 15 cm. Raw sewage alone did not support the growth of *Spirulina*. When seed culture was added to sewage, most of the cells underwent bleaching and finally lysis. Therefore, the effect of fortifying the cultures with different combinations of inorganic carbon, nitrogen, phosphorus, calcium, magnesium, and sodium was tested to determine the algal growth-limiting nutrients in sewage (fig. 1). For comparison, another culture was maintained in parallel supplied with full ingredients of the basal medium. The results demonstrated that *Spirulina* could grow adequately in sewage supplemented with a mixture of bicarbonate and nitrate. The rest of the basal ingredients could be eliminated.

In the attempt to determine the minimal levels of bicarbonate and nitrate, it was found that for outdoor mass culture 1% sodium bicarbonate and 0.1% sodium nitrate must be added to sewage in order to maintain a long-term stabilized biomass production rate. It is not possible to reduce the concentrations of these chemicals below the indicated levels (table 1).

Besides conventional sodium nitrate, various other nitrogen fertilizers, namely, urea, ammonium nitrate, ammonium chloride, ammonium sulphate and diammonium phosphate could also be used to adapt *Spirulina* to sewage. When urea was fed every 15 days at low concentrations, the yield of *Spirulina* was better than the yield obtained when sodium nitrate was used as nitrogen source but the biomass showed reduced protein content (table 2). With ammoniacal nitrogen sources, peak yields as well as the protein values were lower.

When bicarbonate and nitrogen were no longer added to sewage, the algal productivity started to decline after 12-15 harvests. No improvement in growth occurred upon addition of bicarbonate, but when nitrogen was supplied the yield increased dramatically. It was concluded, therefore, that nitrogen was the limiting factor and needs replenishment after about 15 days to maintain the optimal population density for sustained yields.

The optimal culture density in cultivation ponds varies from 75 to 95 g m^{-2} dry weight in summer and from 40 to 60 g m^{-2} in winter. During the period of daily harvesting, the density was not allowed to fall below 35-65 g m^{-2} in order to maintain a minimal algal population required to satisfactorily increase the biomass each day. The cellular density could be kept constant by filtering one-tenth of the total algal suspension each day and returning the filtrate to the

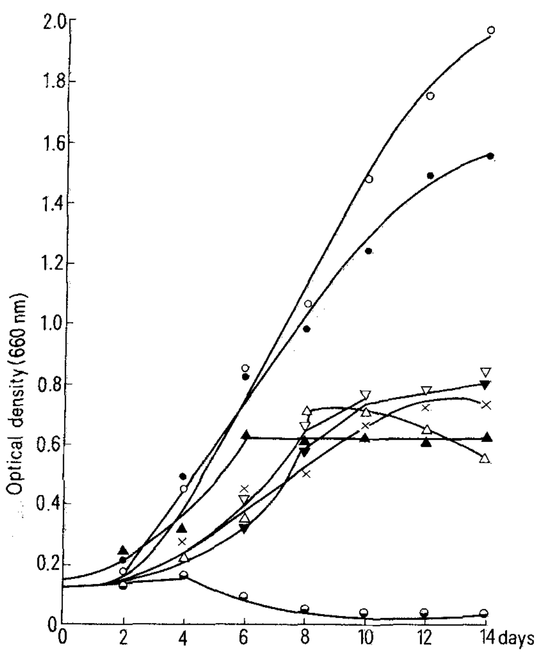

Figure 1. Growth potential of *Spirulina* on sewage. ○, Sewage + all ingredients of the basal medium; ●, sewage + bicarbonate and nitrate; ▽, sewage + nitrate, magnesium and calcium; ▼, sewage + nitrate, phosphate and sodium chloride; X, clean water + bicarbonate and nitrate; ▲, sewage + bicarbonate; △, sewage + nitrate; ◒, raw sewage only.

Table 2. Effect of various nitrogen sources on the growth of *Spirulina* in sewage

N sources	Sodium nitrate	Urea	Ammonium nitrate	Ammonium chloride	Ammonium sulphate	Diammonium phosphate
Optimal concentration (%)	0.1	0.01	0.02	0.02	0.02	0.015
Culture days	12	12	12	12	12	12
OD_{660} (initial)	0.03	0.03	0.03	0.03	0.03	0.03
OD_{660} (final)	1.27	1.30	1.04	0.75	0.63	0.91
Peak yield (g $m^{-2}day^{-1}$)	9.5	9.7	7.8	5.6	4.7	6.8
Crude protein (%)	55	40	36	37	34	30

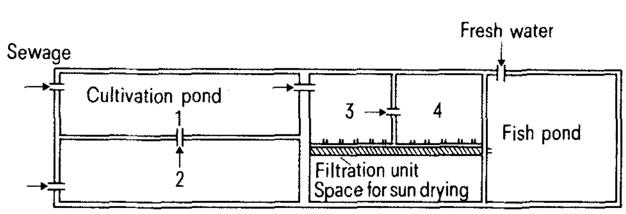

Figure 2. Line diagram of pilot plant for *Spirulina* cultivation in sewage.

ponds. In this manner, a semicontinuous culture with daily harvest was established.

The cultivation process. The method we have developed for cultivating *Spirulina* consists of using domestic raw sewage supplemented with bicarbonate as the carbon source and nitrogen, preferably as nitrate, to increase biomass production. The other essential nutrients are provided by the sewage. The harvesting and processing methods are based on simple filtration and sun-drying. An indigenously designed and constructed functional pilot plant with 450 m^2 surface area has been in operation with a system of daily harvesting for the past 2 years.

The plant, of concrete construction, consists of 4 cultivation ponds (2 of 150 m^2 and 2 of 75 m^2 surface area), a filtration unit based on gradient flow, and a 260 m^2 fish pond containing a polyculture of commercial carp (fig.2). The ponds have a capacity for holding 60,000 l of sewage at 15 cm depth, or 100,000 l at 25 cm operative depth. The filtration unit is a channel of about 4000-l capacity and is 23 m long, 60 cm wide and 30 cm deep. It has iron bars fixed breadthwise on its top to support the cloth screen.

In the cultivation ponds depth is usually maintained at 15 cm during the winter months and 25 cm during the summer by daily addition of sewage. The ponds are agitated by blowing compressed air through aeration pipes for 3-5 min every h. Manual mixing by means of brushes is more practical and does not require electrical energy. For harvesting, one-tenth of the total culture volume is filtered every day, using a polyester cloth. The filtered biomass is repeatedly washed with fresh water, spread thinly on polyethylene sheets and sun-dried.

After each harvest the filtrate is cycled back into the ponds. Nitrate fertilizer is replenished after 15 harvests to maintain optimal algal production.

Peak long-term monthly yields during summer vary between 7.3 and 9.5 g m^{-2} day^{-1}, but decrease to under 5 g m^{-2} day^{-1} during winter. In our conditions the pilot plant is operational for about 9 months out of the year. The culture has to be discontinued during the rainy season which usually lasts from mid-June to mid-September.

Chemical composition. The chemical composition of sewage-grown *Spirulina* is given in table 3. Amino acid analysis showed that most of the essential amino acids fulfil the FAO requirements[14]. The one obvious deficiency is the low level of sulphur amino acids. The samples were essentially devoid of cystine and contained low levels of methionine.

Since the sewage is supplemented with nitrate and the biomass is to be used in poultry feeds the material was examined for nitrosamines. The alga was found to contain 0.04 ppm nitrite ($NaNO_2$) and 2.5 ppm total N-nitrosamine (N-nitrosopyrrolidine). The content of nitrite itself is low and would be unlikely to lead to much of a problem from the synthesis of N-nitrosa-

Table 3. Chemical composition (%) of sewage-grown *Spirulina platensis* sun dried

Moisture	6–7
Ash	8.5–9.9
Crude fiber	0.1–0.9
Crude protein	50–55
Total carbohydrates	18–20
Total lipids	6.5–9.0
Nucleic acids	3.3–4.95
RNA	2.6–4.0
DNA	0.7–0.95
Chlorophyll a	0.76–0.94
Carotenoids	0.22–0.34
Phycocyanin	0.8–1.01
Essential amino acids	
Threonine	5.41
Valine	6.86
Methionine	1.52
Isoleucine	3.91
Leucine	8.30
Tyrosine	2.10
Phenylalanine	2.76
Lysine	3.94
Nonessential amino acids	
Aspartic acid	12.51
Serine	7.21
Glutamic acid	10.44
Proline	5.35
Glycine	7.78
Alanine	9.76
Histidine	1.87
Ammonia	6.14
Arginine	4.06

Table 4. Microbiological analysis of sewage-grown *Spirulina*

Aerobic colony count at 37°C cfu g^{-1}	3×10^5 [a]
Anaerobic colony count at 37°C cfu g^{-1}	6×10^5 [b]
Moulds: colony count at 25°C cfu g^{-1}	7×10^3
Clostridium perfringens cfu g^{-1}	$< 1 \times 10^2$ [c]
Bacillus cereus cfu g^{-1}	$< 1 \times 10^2$
Total Enterobacteriaceae cfu g^{-1}	$< 1 \times 10^2$
Escherichia coli type 1 MPN g^{-1}	0.4
Presumptive faecal streptococci cfu g^{-1}	2×10^5
Salmonellae in 50 g	Not detected
Shigellae in 50 g	Not detected
Vibrio cholerae or other vibrios in 100 g	Not detected
Aeromonas in 0.1 g	Not detected

[a] Predominantly *Bacillus* sp.; [b] predominantly catalase-negative cocci, possibly faecal streptococci; [c] other clostridia present in 0.001 g sample; cfu, colony forming units; MPN, most probable number.

Table 5. Effect of replacing groundnut cake with sewage-grown *Spirulina* on growth and feed efficiency of growing chicks

Groundnut cake	*Spirulina* (% of ration)	6-week gain* (g)	Feed consumed (g)	Feed efficiency (g gain g^{-1} feed consumed)
24	0	172.7 ± 5.1^b	652 ± 12.0	0.26
16	5.6	175.7 ± 5.0^b	643 ± 1.5	0.27
8	11.1	195.9 ± 6.6^a	662 ± 9.5	0.29
0	16.6	193.8 ± 5.7^a	666 ± 14.0	0.29

*Values with unlike superscripts are significantly different ($p < 0.01$).

mines in vivo on ingestion. Furthermore, it is likely that alternative chicken feeds would have contents of total N-nitroso compounds of this order, particularly if they were based on fish meal.

Microbiology and parasitology. In view of the growth substrate (human sewage supplemented with bicarbonate and nitrate) and the production conditions (aerated, stirred, and exposed to intense sunlight) it was considered advisable to examine the material for its general microbiological condition and for some of the bacteria likely to be present in human sewage and/or to cause food borne infections or intoxications. Crude sewage contains all the agents causing infectious disease in man – bacteria, protozoa, viruses, intestinal parasites – excreted through the intestinal tract. Algae cultured on crude sewage might be expected to be contaminated with many of these organisms unless the cultural conditions are such that the organisms do not survive. This appears to be the case in our pilot plant since the samples examined were in unexpectedly good microbiological condition. Many vegetative organisms would be sensitive to the highly alkaline conditions, to prolonged exposure to intense sunlight, to continuously high ambient temperature, to drying, and to the combination of these factors.

The flora isolated appeared to consist predominantly of gram-positive spore-forming bacilli and (presumptive) faecal streptococci; clostridia were present in small numbers (table 4). With the exception of very low numbers of *Escherichia coli* type 1 (either survivors from sewage or harvesting/post-harvesting contamination) no Enterobacteriaceae were detected. *E. coli* is a normal inhabitant of the intestine of humans and most warm-blooded animals. It is an opportunistic pathogen and its presence is considered an indication of faecal contamination. No pathogenic forms like *Salmonella* and *Shigella* were present. Similarly the members of the family Vibrionaceae, such as *Vibrio cholerae* or other vibrios, and *Aeromonas* were absent. We have not attempted to detect *Aeromonas* in larger amounts than 0.1 g since small numbers of *Aeromonas* would be quite insignificant. Most animals probably drink several thousand or even millions of *Aeromonas* each day.

It is unlikely that faecal streptococci would be a problem in poultry feed because these organisms are, in any case, very early colonizers of the chick gut. Faecal streptococci grow in the presence and absence of air and are fairly tolerant of alkaline conditions. Also, they tend to survive in conditions which inhibit many other vegetative organisms, although they are not as resistant toward unfavorable conditions as endospores of *Bacillus* and *Clostridium*.

Bacillus species are aerobic although some strains will also grow in the absence of air. Clostridia are anaerobic and are mostly very sensitive to oxygen. The spores of both bacilli and clostridia may be very resistant to adverse conditions. In view of the strongly aerobic cultural conditions of the alga the presence of fairly high levels of *Bacillus* species is not unexpected, although their source is more likely to be adventitious contamination rather than the sewage substrate. *Bacillus* spores are common contaminants of dry natural products. Growth or survival of vegetative cells of clostridia would not be expected in an environment subject to constant aeration. However, the resistant spores would be likely to survive. Gram-negative bacteria such as the Enterobacteriaceae and Vibrionaceae tend to be quite susceptible to UV-light and heat and, therefore, are expected to die during the drying process.

Of the toxigenic and pathogenic organisms, *Bacillus cereus* and *Clostridium perfringens* were found to be present. *B. cereus* can cause mild food-borne intoxications in humans. *C. perfringens* is the most common pathogen of sheep and occasionally it may affect man and other animals. In man it may be responsible for food poisoning and wound infections. *Spirulina* biomass contaminated with these toxigenic bacteria was used in the chick and laying hen feeding trials reported in this paper. In no case has there been evidence of toxicity or other adverse reaction. We do not, however, know of studies where these organisms have been fed to poultry at low levels over an extended period of time, or the consequences thereof.

The dried algal samples were negative for *Entamoeba histolytica.* Among the aerobic free-living amoebae, only *Acanthamoeba* could be isolated from the samples inoculated on non-nutrient agar plates seeded with *E. coli.* Its occurrence was rare, however. Whereas cysts of *E. histolytica* are highly sensitive to desiccation, those of *Acanthamoeba* are quite resistant and are likely to be present in the material. Although certain species of *Acanthamoeba* are known to cause meningo-encephalitis in humans and animals, clonal cultures of amoebae present in our samples were tested in mice intranasally and proved to be non-pathogenic.

To examine the possibility of enteroviruses, the samples were inoculated into cultures of baboon kidney and passaged after 1 and 2 weeks into human embryo kidney and lung diploid cell cultures. No cytopathic effects have been detected in any cultures and no virus particles could be seen by electron microscopy. There is therefore no evidence of enteroviruses in the algal product.

The material was also tested for the presence of worm cysts, but none were found.

Feeding experiments. Growth studies. Sewage-grown *Spirulina* was used as a substitute for groundnut cake in feed for White Leghorn chicks. The control ration was based on groundnut cake having 38% protein and contained the following ingredients in g% of diet:

Yellow maize 40.0; rice bran 19.0; molasses 4.5; groundnut cake 24.0; meat and bone meal 10.0; vitamin and salt mixture 2.5. *Spirulina* replaced the groundnut cake in experimental diets on an equal-nitrogen basis at levels of 5.6, 11.1 and 16.6%.

Each of the 4 diets was given to batches consisting of 30 chicks. The chicks were fed ad libitum. The increase in weight and the consumption of feed were recorded daily for 6 weeks.

The data presented in table 5 illustrate the effect of using *Spirulina* in place of groundnut cake on growth and feed efficiency of chicks. Although slightly lower, the body weight of the control batch showed no significant difference with the experimental batch on 5.6% *Spirulina*. However, chicks fed experimental diets containing 11.1 and 16.6% *Spirulina* did reveal significantly higher weight-gains as compared to the controls.

The batch treated with *Spirulina* at 5.6% level ingested slightly smaller amount of feed and the chicks fed 11.1 and 16.6% *Spirulina* consumed slightly larger amounts than the controls, without this however being significant. Feed efficiency was also similar in the case of the control and *Spirulina*-fed batches.

There was no mortality in any of the batches during the course of the experiment and no abnormalities were noted in the experimental chicks indicating that *Spirulina* produced in the sewage system is nontoxic.

These studies show that sewage-grown *Spirulina* can totally replace groundnut cake, the chief source of vegetable protein supplement in starter poultry feeds.

Egg yolk pigmentation studies. The degree of yolk color in table eggs is subject to consumer preferences which vary widely throughout the world. In India, golden yellow colored yolks with a definite orange tinge are generally preferred due to which a premium of up to 50% is routinely obtained in the retail market for indigenous eggs as compared to lightly pigmented farm-produced eggs.

The poultry industry depends on yellow maize and dehydrated berseem meal as major pigmentation sources. Yellow maize, the main source of pigments, constitutes about 40% in layer diets. With the fast expanding poultry industry in the country the demand for yellow maize has steadily increased with the result that its market price has gone up considerably. The increased usage of damaged wheat and rice as yellow maize substitutes has further accentuated the difficulty of producing adequately pigmented egg yolks for table use. The pigmenting efficacy of sewage-grown *Spirulina* for egg yolks was assessed in laying hen trials.

The control ration (T1) contained the following ingredients in g% of diet: Damaged wheat 30.0; damaged rice 35.0; groundnut cake 12.0; meat cum bone meal 9.0; molasses 7.0; chalk 5.0; vitamin and salt mixture 2.0. *Spirulina* at levels of 3, 6, 9, 12, 15, 18 and 21% was added in place of groundnut cake in treatment rations T2 to T8; yellow maize was included at levels of 10, 20, 30 and 40% replacing damaged wheat and part of damaged rice in treatment rations T9 to T12; dehydrated berseem meal at 5 and 7.5% levels was incorporated in treament rations T13 and T14 at the expense of the control ration. The diets T2–T8 contained neither yellow maize nor dehydrated berseem meal; hence, *Spirulina* was the sole supplier of pigments.

The experiment with laying birds was performed with White Leghorn hens housed in 3-tier individual cages. 42 selected layers were divided into 14 groups of 3 hens each. Feeding of the test diets began after a preparatory period of 12 days during which the hens were fed the control ration in order to bring about depletion of carotenoids in the birds. Thereafter, the

Table 6. Visual yolk pigmentation scores of different treatment groups and indigenous eggs

Treatments	Raw eggs	Boiled eggs
T 1 – Control	1.5 ± 0.01	1.0 ± 0.00
Sewage-grown *Spirulina*		
T 2 – 3%	13.3 ± 0.16^{a}	9.0 ± 0.13^{a}
T 3 – 6%	13.0 ± 0.12	9.3 ± 0.13
T 4 – 9%	13.1 ± 0.22	11.9 ± 0.02
T 5 – 12%	14.2 ± 0.21	12.9 ± 0.01
T 6 – 15%	14.4 ± 0.15	13.0 ± 0.00
T 7 – 18%	14.1 ± 0.22	12.9 ± 0.07
T 8 – 21%	14.8 ± 0.11	13.4 ± 0.14
Yellow maize		
T 9 – 10%	4.7 ± 0.30	2.3 ± 0.13
T10 – 20%	6.6 ± 0.15	3.0 ± 0.16
T11 – 30%	7.4 ± 0.26	4.3 ± 0.14
T12 – 40%	8.0 ± 0.24^{c}	6.2 ± 0.12^{c}
Dehydrated berseem meal		
T13 – 5%	4.8 ± 0.26	2.4 ± 0.14
T14 – 7.5%	5.6 ± 0.26^{d}	3.9 ± 0.17^{d}
T15 – Indigenous eggs	10.8 ± 0.89^{b}	7.0 ± 0.25^{b}

[a,b,c,d] Values in the same column with unlike superscripts are significantly different ($p < 0.01$).

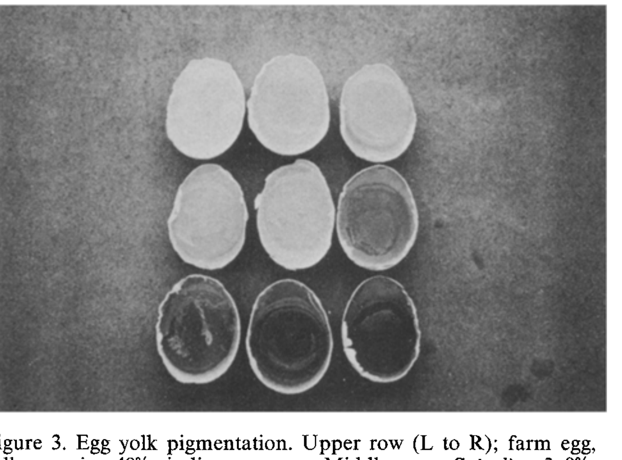

Figure 3. Egg yolk pigmentation. Upper row (L to R); farm egg, yellow maize 40%, indigenous egg: Middle row; *Spirulina* 3–9%, *Spirulina* 12–18%, *Spirulina* 21%; Bottom row; egg yolks produced by hens fed *Spirulina* at levels above 21%.

14 groups were assigned to each of the dietary treatments. The different diets were fed ad libitum. The experiment was allowed to run for 30 days. Individual egg production records were kept daily throughout the experiment. Eggs obtained from all the treatment groups and indigenous eggs (T15) were subjected to a yolk color test. Visual color scores of egg yolks were estimated independently by 3 individuals using the color fan of Roche as a reference.

Hens previously maintained on a oxycarotenoid-free feed (control ration) and depleted of their carotenoids transferred dietary pigments to the egg yolk within 72 h after being fed *Spirulina*-containing diets. Yolk color intensity reached a maximum after 7 days.

The effects of various test diets on yolk pigmentation of eggs are presented in table 6. The results show *Spirulina* to be an excellent yolk pigmenter. The visual scores of the yolks produced by the birds fed *Spirulina* diets were markedly higher (13.0–14.8) than those obtained from birds fed yellow maize (4.7–8.0) and dehydrated berseem meal (4.8–5.6). Indigenous eggs showed a higher Roche fan score (10.8) for yolks than yellow maize and berseem meal groups, but *Spirulina* gave the highest readings at all levels and produced much deeper yolk color than that produced by the highest levels of the 2 conventional oxycarotenoid sources. Feeding of 3% *Spirulina* produced deep yellow-orange yolks that scored 13.3 on the Roche yolk color fan. 12% *Spirulina* imparted a brilliant reddish-orange color to the egg yolks with a visual score of 14.2 which increased to an average of 14.8 with the algal additions up to 21%, but yolk pigmentation did not increase in proportion to dietary algal concentration.

The difference of yolk color between treatments T2 (3% *Spirulina*), T12 (40% yellow maize), T14 (7.5% dehydrated berseem meal) and T15 (indigenous eggs) was statistically significant ($p < 0.01$).

A similar trend was noticed in boiled eggs which, however, showed lower yolk color values than raw eggs. This might be due to the brightness of the vitelline membrane.

In a consumer preference study with hard boiled eggs most of the judges preferred table egg yolk colors having a Roche fan score between 9 and 12. These colors corresponded to feeding *Spirulina* at levels of 3–9% in layer diets.

The efficiency of *Spirulina* yolks for imparting suitable color to cakes was also determined. It was observed that the pigments in the alga carry over well into cakes via the egg yolks from hens fed *Spirulina* meal supplement. Sponge cakes made with eggs from hens receiving 3% *Spirulina* showed a light yellow-orange tinge. 12% *Spirulina* eggs imparted a definite golden-yellow color to the cakes and 21% produced such deeply pigmented yolks that the cake was deep yellow with red overtones.

In India, there is no distinction between the eggs meant for table use and 'breaker eggs' required for the commercial production of bakery and other egg-containing products in which a particular depth of color is desired and must be provided by egg yolk rather than by addition of artificial coloring agents. A sizable percentage of the total egg production in this country is utilized in bakery products and obviously highly colored egg yolks would be preferred by the food processing industry.

Our studies have shown that sewage-grown *Spirulina* can be used as the sole yolk pigmenter to obtain satisfactory yolk color in the absence of yellow maize and dehydrated berseem meal, both of which can be totally replaced with white maize in layer rations. At modest levels *Spirulina* produces deeply colored yolks for table use; higher levels produce heavily pigmented orange-red yolks that have a satisfactory carry-over in cakes and, therefore, would be most suitable for the bakery industry.

The wide range of pigmentation potential resulting from the use of *Spirulina* as the sole source of pigments would enable the egg producer to impart the desired degree of color to yolks and to obtain eggs meant exclusively for table use or for commercial purposes (see fig. 3).

Pisciculture. Pond effluent mixed with fresh water is used for rearing commercially important species of carp, such as *Cyprinus carpio*, *Labeo rohita* and *Cirrhinia mrigula* as a by-product of the waste recycling system. The fish are fed a diet composed of *Spirulina* powder 5%, mustard cake 15% and rice bran 80%. The production of fish is about 500 kg/year.

Irrigation water. The characteristics of an irrigation water that are most important in determining its quality are the total concentration of soluble salts and the relative proportion of sodium to other cation

Table 7. Fish pond water characteristics

Month	meq l^{-1} Ca^{++}	Mg^{++}	Na^{+}	K^{+}	Total cations	CO_3^{--}	HCO_3^{-}	SO_4^{--}	Cl^{-}	Total anions	Total dissolved solids (ppm) (TDS)	Sodium adsorption ratio (SAR)
January	1.50	0.70	4.70	0.60	7.50	0	3.20	4.30	0.80	8.30	510	4.4
March	1.10	0.50	4.10	0.40	6.10	0	2.75	2.17	0.90	5.82	380	4.6
June	1.75	1.10	5.40	0.50	8.75	0	2.59	4.56	1.30	8.45	586	4.5
September	0.90	0.40	3.39	0.21	4.90	0	1.90	2.10	1.40	5.40	310	4.2
November	1.20	0.90	4.82	0.76	7.68	0	3.60	3.10	0.45	7.15	506	4.7

concentrations. In general, irrigation water in which the total dissolved solids (TDS) are below 500 mg l^{-1} may be used on all but the most sensitive crops. Provided that leaching and drainage are adequate, TDS concentrations of between 500 and 1500 mg l^{-1} may be and are widely used[20]. The relative concentrations of sodium to those of calcium and magnesium affect the alkaline nature of the soil as sodium has a tendency to replace calcium and magnesium in the soil. Irrigation water with a low mineral content may be used with a sodium adsorption ratio (SAR) up to 26.0[5].

With reference to table 7, the fish pond's water shows TDS ranging from 310 to 586 ppm and SAR values between 4.2 and 4.7. It may be classified as being medium in dissolved solids, as having low SAR values and is satisfactory for use on almost all soils.

Conclusions. The technology of *Spirulina* biomass production in domestic sewage has become a reality with the development of a prototype by us. The twin advantages of this system are a) reclamation of sewage wastes through biological oxidation by the alga, and b) production of algal biomass which can be used as a source of protein and pigments for poultry. Together with fish-culture and irrigation water, the whole process offers a complete package.

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