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Author(s) PROSPERI A., BERNAGOZZI M., ROMANO G.C. & TONELLI E.

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Application of algal bioassays in the determination of eutrophic power of waste water.

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The term "eutrophic" which traditionally refers to a natural and optimal condition of a water-body, nowadays has come to mean instead a state of pollution due to an excess of nutritive substances accompanied by an increased production of algal microflora. Eutrophication is a process tied up with transfer of nutrients, natural or artificial in the surface water which, may exert a beneficial effect when within the limits suited to the biological capacity of the receiving water-body whereas, in excess, can cause a pollution, (Genovese 1973).

The best evidence for establishing the level of eutrophy of a water-body is its algal production which makes it possible to identify the type and the intensity of the eutrophication according to the kind and number of algal species present: when the number of algae exceeds half a million per litre then one speaks of an "algal bloom". (Fruh, 1967).

Eutrophication causes a notable increase in the total amount of algae, represented however by few species: on the contrary in oligotrophic waters, that is, those low in nutrients one observes a relatively modest growth of aquatic microorganisms but with many kinds of species.

An increase in productivity is thus secondary to an increase of nutrients. These, indeed, among which we note especially nitrogen and phosphorus, are considered to be the essential cause, and fundamental in acceleration, of eutrophication (Sawyer 1947, Weiss 1969, Gerloff et al 1957).

Phosphorus in particular has been shown from numerous experiments to be the most important element. At the start of the process other substances also participate such as potassium, magnesium, sulphate, traces of metals and numerous organic micro nutrients. (Provasoli 1961: Goldmann 1965).

Some workers take 2.8 µg/l. of phosphorus as the maximum amount in unpolluted water (Ketchum 1969): others record that algal blooms occur when the mean annual concentrations of phosphorus and nitrogen are 0.015 and 0.3 mg/l respectively (Sawyer et al 1944).

Some authors think that carbon, in the form of "carbonic anhydride" produced in bacterial decomposition of organic waste (Kuentzel 1969) or in the oxidative metabolism of heterotrophic populations (Kerr et al 1970), can play an important part in promoting development of noxious algae, thus relegating direct and active absorbtion of phosphorus and nitrogen to a secondary role. Moreover, whether or not both are nutrient limiting factors, there is good reason to believe that in reality the situation is even more complex than these views suggest and that in fact numerous elements interact to determine the productivity of a water-body and its trophic state.

Among the factors that influence the enrichment by nutrients of the harvest in superficial waters, one should consider some geological factors; nutritional transport in some cases may depend on the geochemistry of the drainage basin (possibilities for percolation and washing of the soil and rocks), the size of the basin and of its hydrology (Brezonik 1972).

It is necessary to record also that the productivity of a water-body may be affected by factors other than the concentration of nutrients, factors, for the most part unknown, which condition the distribution, the availability and the use of the same nutrients.

For these reasons, the traditional chemical, physical and bacteriological indices are not sufficient for a true evaluation of the trophic capacity of a water and in fact we find that instead the most effective method is the determination in the laboratory of algal growth applying the procedures of the biological sages propounded in the "National Eutrophication Research Program" (1971).

These methods enable one to observe directly the influence of the water in tests on the development either of indigenous algae or of selected "experimental" algae (Selenastrum capricornutum, Microcystis aeruginosa, Anabaena flos-aquae) thus determining biologically the availability of limiting nutrients for the growingalgae.

Moreover, by artificially increasing those nutrients in reduced concentrations, one can use the test algae to identify which are the limiting factors and what concentrations are needed to produce an algal bloom. In this way it is possible to forecast with some precision the potential effect on the development of the algae on addition of nutrients. This last possibility is of great value because one can indicate, even in ways not wholly exact, that a water-body is becoming progessively more eutrophic and to what extent. For an evaluation of this question continuous and periodic uniform bioassays will be necessary as well as other routine chemical and physical tests.

Sanitary authorities and many students of the problem are interested in eutrophication because the phenomenon in question is related to the serious deterioration of the quality of water (disagreeable tastes and odours, particle colouring, intense turbidity etc.) thus rendering it unfit for other uses, dometic, industrial, agricultural, and recreational; and afterwards very difficult and inconvenient to eliminate with modern and expensive treatment (Fruh 1967).

Among the more important causes of the progressive enrichment in nutrient substances of the water are in particular the indiscriminate use of phosphate detergents, intensive agriculture use of fertilisers, industrialisation, and urbanisation of populations; consequently through direct discharge of sewage, one has a strong and continuous contamination of the superficial water-table.

Primary and secondary treatment, to which domestic discharges are normally subjected, if on the one hand they get rid of hazardous decompositions and also to some extent those which are infective, on the other they increase the capacity for eutrophication in many forms especially oxides of nitrogen and phosphorus which are the most efficient stimulants of the growth of algae (Middlebrooks et al 1971).

It becomes necessary therefore in most instances to adopt a third kind of treatment which, amongst various methods(Bernard 1972) is capable of removing almost all the content of fertilizer substances (Miller et al 1971).

The scope of the present research aims to: verify if the Selenastrum capricornutum can be used as a test alga under our culture conditions * :to determine the eutrophic level of the secondary effluent of a modern plant for the treatment of domestic discharge and to investigate the eventual "limiting factors"; and to study the effect on the secondary effluent of testiary treatment carried out artificially in the laboratory.

Material and methods.

Water samples for experiments were taken from the outflows of a purification station in our province. The installation, of recent design and working, purifies water of exclusively domestic origin and is based upon our traditional system of primary and secondary treatment. This last is formed from an activated sludge treatment plant.

The effluent was collected at four distinct times of year:winter (Feb. 1974) spring (May 1974), summer (July 1974) and autumn (September 1974). On the spot, at the moment of sampling, we have measured dissolved oxygen and the temperature of the water-samples and of the air. We have taken note of the prevailing atmospheric conditions and those for the preceding days.

On each sample we have made chemical tests and biological trials for algae (see table).

Chemical analysis was carried out according to the methods suggested in "Standard methods for the examination of water and wastewater"(1971), the test water being previously filtered through filter paper Whatman no. 1. This was done with all samples and has been repeated also in the laboratory after application of the third treatment.

These last have been carried out on the second, third and fourth samplings using respectively ammonium sulphate (200mg./l) calcium oxide (700 mg/l)and aluminium sulphate (200 mg./l) with successive filtrations through activated charcoal (Bernard 1972).

Each sample, after chemical estimations has been subjected to algal bioassay according to the proceedings outlined in the "Provisional algal assay Procedure" (National Eutrophication Research Program, 1971) using exclusively as test species the unicellular green alga, Selenastrum capricornutum Prinz.

At the beginning of the bioassays, all samples were filtered through membrane filters millipore of 0.46μ to retain all algae present, and then stopped and inoculated at pH 7. The tests were made in 250 ml glass-bottles holding 60 ml of the test water. All the samples subjected to third treatment have been made up to, either singly or in phosphorus combination (as K_2HPO_4), to a concentration

* We thank the National Eutrophication Research Program, Pacific North West Water Laboratory, Corvallis, Oregon (U.S.A.) for having kindly supplied this laboratory with the unicellular green alga Selenastrum capricornutum Prinz.

CARATTERI CHIMICO-FISICI E CRESCITA ALGALE NELLE ACQUE ESAMINATE

DETERMINAZIONI CHIMICO-FISICHE	*campione del 21-2-1974*	*campione del 2-5-1974*		*campione del 27-7-1974*		*campione del 28-9-1974*	
	effluente secondario	*effluente secondario*	*effl. second. trattato con* $Al_2(SO_4)_3$ *(200 mg/l)*	*effluente secondario*	*effl. second. trattato con CaO(700 mg/l)*	*effluente secondario*	*effl. second. trattato con* $Al_2(SO_4)_3$ *+ Carbone attivato*
Kjeldahl - N (mg/l)	1,56	1,79	0,89	2,12	1,34	1,34	0,78
Ammoniaca - N (mg/l)	0,95	20,20	18,00	6,40	0,80	2,16	0
Nitriti - N (mg/l)	0,21	0,016	0,078	0,116	0,145	0,027	0,123
Nitrati - N (mg/l)	18,00	2,00	1,10	8,40	8,00	4,20	3,00
C.O.D. (mg/l)	52,81	67,84	49,70	21,03	8,20	18,50	0
Sostanze organiche (mg/l O_2 consumato)	10,40	10,40	8,40	6,30	6,00	5,00	2,80
Ortofosfati - P (mg/l)	0,233	0,202	0,023	0,132	0,0007	0,111	0,0012
Fosfati totali - P (mg/l)	0,300	0,300	0,046	0,146	0,0014	0,145	0,003
Cloruri (mg/l)	71,60	90,40	88,62	59,50	54,90	96,10	102,80
Alcalinità (mg/l $CaCO_3$)	0,248	0,330	0,264	0,322	0,582	0,360	0,256
Durezza totale (°F)	29	32	29	34	63	38	40
pH	7,40	7,60	7,85	8,00	11,85	8,25	8,20
Detergenti (mg/l)	0,240	0,216	0,304	0,104	0,124	0,130	0,045
Conducibilità elettrica (μS/cm)	700	735	775	675	2.025	892	875
Ossigeno disciolto (mg/l)	8,20	5,50	—	6,60	—	7,40	—
Temperatura acqua (°C)	8,00	18,00	—	21,00	—	22,00	—
Temperatura atmosferica (°C)	12,00	20,50	—	23,50	—	25,00	—
CRESCITA ALGALE in cellule/ml (inoculo $1 \cdot 10^3$ cellule/ml)							
Dopo 10 giorni	$3,4 \cdot 10^4$	$7,6 \cdot 10^4$	$4,1 \cdot 10^4$	$2,4 \cdot 10^4$	$3,0 \cdot 10^3$	$2,1 \cdot 10^5$	$7,0 \cdot 10^3$
IDEM dopo aggiunta dei nutrienti in mg/l							
+ 10,0 C	$3,5 \cdot 10^4$	—	$3,8 \cdot 10^4$	—	$3,0 \cdot 10^3$	—	$3,2 \cdot 10^4$
+ 1,0 N	$3,8 \cdot 10^4$	—	$3,2 \cdot 10^4$	—	$3,1 \cdot 10^3$	—	$5,8 \cdot 10^4$
+ 0,05 P	$3,6 \cdot 10^4$	—	$7,7 \cdot 10^4$	—	$7,2 \cdot 10^3$	—	$1,1 \cdot 10^5$
+ 10,0 C + 1,0 N	$4,0 \cdot 10^4$	—	$3,3 \cdot 10^4$	—	$5,0 \cdot 10^3$	—	$3,8 \cdot 10^4$
+ 10,0 C + 0,05 P	$3,7 \cdot 10^4$	—	$7,9 \cdot 10^4$	—	$9,2 \cdot 10^3$	—	$5,0 \cdot 10^4$
+ 1,0 N + 0,05 P	$3,8 \cdot 10^4$	—	$6,8 \cdot 10^4$	—	$1,0 \cdot 10^4$	—	$1,7 \cdot 10^5$
+ 10,0 C + 1,0 N + 0,05 P	$3,9 \cdot 10^4$	—	$7,8 \cdot 10^4$	—	$1,1 \cdot 10^4$	—	$9,9 \cdot 10^4$

of 0.05 mg/l; nitrogen (as Na NO3) to 1.0mg/l and carbon (as NaHCO3) to 10.0mg/l (Maloney et al 1972).

Each bioassay has been repeated three times starting with an inoculation of $Ix10^3$ cells/ml: the test alga has been cultured in the lab. in modified soil PAAP (Murray et al 1971).

All samples were incubated for 21 days at thermostatically controlled temperature of 22± 2°C, and subjected to a cold light unbroken of 4300 lux and continually agitated on a small table rotating at 110 oscillations per minute.

The growth of the alga was found daily by direct count under the microscope using the (camera Thoma contaglobuli)* . The values given in the tables and in the text are for time of maximum development (10°day of incubation) and are the mean of three observations made on each sample.

Results

From study of the obtained results (see Table) one can see that samples taken in September generate an algal development notably superior to that observed in the other periods, not withstanding an inferior content of phosphorus and nitrogen.

The eutrophic power of the secondary effluent has been constantly reduced by tertiarytreatment applied in the laboratory which has assured the constant reduction of phosphorus either as total phosphorus or other forms of orthophosphate in all the samples.

In particular, addition of calcium oxide to the secondary effluent with drawn in summer has yielded a reduction in total nitrogen of 37% and has cut down total phosphorus by as much as 99%.

With bioassays made after this kind of treatment and after stoppering the samples at pH 7, the resulting response of algal development was notably inferior to the growth-values found in the secondary effluent: one finds in fact from 2.4×10^4 cell/ml to 3.0×10^3 cells/ml in the water after treatment.

One proceeds then to add to the tertiary effluent the principle nutrients in order to decide which are the limiting factors.

Phosphorus already present at a concentration of 7×10^{-4} mg./l. in orthophosphates, when added artificially to the test water about doubles the level of algal growth. Moreover carbon and nitrogen added in combination with phosphorus produce a threefold increase of algae. These results show that phosphorus limits the growth of algae; carbon and nitrogen become limiting when phosphorus is present in sufficient quantity.

The water of the secondary effluent collected in spring has been subjected in the laboratory to tertiary treatment by adding aluminium sulphate. As the literature indicates, this method reduces effectively the major elements concerned in eutrophication namely nitrogen, phosphorus and (oligoelements?).*

* Translation uncertain.

* Translation uncertain.

From study of the chemical analyses, one sees that total phosphorus is reduced by about 1.85% + total nitrogen by 50%. The growth-response of algae cultured in untreated effluent was 7.6×10^4 cells/ml whereas the same subjected to treatment was 4.1×10^4 cells/ml.

Also in this sample the artificial increase of nutrients has shown that phosphorus is the principle limiting element: in fact addition of phosphorus roughly doubles the rate of algal growth.

The final type of treatment applied in the laboratory to the sampled water in September and consisting of the addition of aluminium sulphate and successive passages through activated carbon produces an improvement in the chemical character of the treated effluent referable in particular to the orthophosphate which in the end is reduced by 99%, total nitrogen by 42% and organic substances by 44%.

Most sensitive to this general abatement are those algae which reached a growth level of 7.0×10^3 cells/ml against 2.1×10^5 cells/ml developed in the effluent treated first.

Analogously to the preceeding samples the added phosphorus shows a very clear increase in the development of Selenastrum capricornutum (1.1×10^5 cells/ml.) when the phosphorus and the nitrogen are added together. The nitrogen then is not limiting the growth in the treated effluent except after the addition of a quantity in excess of phosphorus.

Conclusions.

The results presented do not always show a correlation between development of algae and concentration of nutrient substances. Indeed, the samples obtained in September gave rise to an algal growth (2.1×10^5 cells/ml) notably superior to samples taken in February (3.4×10^4 cells/ml.), May (7.6×10^4 cells/ml) and July (2.4×10^4 cells/ml) though being inferior in content of phosphorus and nitrogen.

Other factors also limiting and correlated with algal multiplication such as dissolved oxygen and C.O.D. do not seem to have influence.

In the reduction of total phosphorus, aluminium sulphate (200 mg/l) has been shown to be the least effective among the three systems of tertiary treatment with an efficiency of 85%, inferior to that of calcium oxide (700 mg/l) and also to aluminium sulphate in conjunction with post-filtration through activated carbon: these last systems have indeed eliminated 98 - 99% of total phosphorus.

Total nitrogen was reduced to much the same extent by all three methods. Algal bioassay, carried out before and after the third treatment indicates the extent of reduction of the eutrophic powers of the secondary liquid in noteworthy manner: in this respect, it is interesting to note how the two treatments which brought about the almost total elimination of phosphorus are also the most effective in reduction of eutrophic power. Indeed algal productivity is reduced by 7 and 40 times respectively by the calcium oxide and by the aluminium sulphate with post-filtration, whilst it is only halved by aluminium sulphate.

Finally, artificial addition of phosphorus, nitrogen and carbon, alone or in combination, to the tertiary liquid has made it possible to single out phosphorus as the limiting factor: nitrogen can become limiting only in the presence of phosphorus in excess.

BIBLIOGRAFIA

BERNARD J.: *Les traitements tertiaries des eaux usées.* Tec. Scienc. Municip., *4*, 145, 1972.

BRUZONIK P.L.: *Cultural Eutrophication: the Nature of Problem and Some Recomendations for its Study and Control.* In « Air and Water Pollution », Ed Adam Hilger - London, 1972.

FRUH E.G.: *The overall picture of eutrophication.* Jour. Water Poll. Control Fed., *39*, 1449, 1967.

GENOVESE S.: *Eutrophication: Récentes orientations pour de nouvelles perspectives.* Rev. Intern. Océanogr. Med., *29*, 53, 1973.

GERLOFF G.C., SKOOG F.: *Nitrogen as a Limiting Factor for the Growth of Microcystis aeruginosa in Southern Wisconsin Lakes.* Ecology, *38*, 556, 1957.

GOLDMAN C.R.: *Micronutrients Limiting Factors and Their Detection in Natural Phytoplancton Populations.* In « Primary Productivity in Aquatic Environments ». Mem. Ist.-Ital. Idrobiol. 18 Suppl. Univ. of California Press, Berkeley, 1965.

KERR P.C., PARIS D.E., BROCKWAY D.L.: *The Interrelation of carbon and phosphorus in regulating heterotrophic and autotrophic populations in aquatic ecosystem.* Water Poll. Control Res. Series 16050 FGS 07/70 U.S. Dep. Interior FWQA U.S. Govt. Printing Office, 1970.

KETCHUM M.B.: *Eutrophication of estuaries.* In « Eutrophication, causes, consequences, correctives ». Nat. Acad. Sci., Washington, 1969.

KUENTZEL L.B.: *Bacteria, carbon dioxide and algal blooms.* Jour. Water Poll. Control Fed., *41*, 1737, 1969.

MALONEY T.E., MILLER W.E., SHIROYAMA T.: *Algal responses to nutrient additions in natural waters. I° Laboratory Assays.* In « Nutrients and Eutrophication », Special Symposia, *1*, 134, 1972.

MIDDLEBROOKS E.J., PORCELLA D.B., PEARSON E.A., MC GAUHEY P.H., POHLICH G.A.: *Biostimulating and algal Growth kinetics of Wastewater* - Jour. Water Poll. Control Fed., *43*, 454, 1971.

MILLER W.E., MALONEY T.E.: *Effects of secondary and tertiary wastewater effluents on algal growth in a lake-river system.* Jour Water Poll. Control Fed., *43*, 2361, 1971.

MURRAY S., SCHERFIG J., DIXON P.S.: *Evaluation of algal assay procedure PAAP Batch Test.* Jour. Water Poll. Control Fed., *43* 1991, 1971.

NATIONAL EUTROPHICATION PROGRAM. ENVIRONMENTAL PROTECTION AGENCY.: *Algal Assay Procedure « Bottle Test ».* U.S. Government Printing Office, 1971.

PROVASOLI L.: *Micro-Nutrients and Heterotrophy as Possible Factors in Bloom Production in Natural Waters.* In « Algae and Metropolitan Wastes », R.A. Taft San. Eng. Center, Cincinnati, Ohio, TR W61-3, 1961.

SAWYER C.N.: *Fertilization of lakes by Agricultural and Urban Drainage.* Jour. New. England Water Works Assn., *61*, 109, 1947.

SAWYER C.N., LACKEY J.B., LENZ A.T.: *Investigations of the odor nuisance occurring in the Madison lakes.* Report of the Governor's Committee, State of Wisconsin, 1944.

STANDARDS METHODS FOR THE EXAMINATION OF WATER AND WASTEWATER. 13 th edition, APHA, AWWA, Water Poll. Control Fed., 1971.

WEISS C.M.: *Relation of Phosphates to Eutrophication.* Jour. Amer. Water Works Assn., *61*, 387, 1969.

Notice

Please note that these translations were produced to assist the scientific staff of the FBA (Freshwater Biological Association) in their research. These translations were done by scientific staff with relevant language skills and not by professional translators.