

Simple electric powered plankton wheel for the production of aggregates in seawater on-board ship

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A new handy plankton wheel was devised for use on-board a ship. Its efficacy to generate aggregates was tested on land using coastal waters. The device successfully generated aggregates of sizes varying up to 11.3 mm² within eleven days. During an on-board experiment with oceanic water within approximately the same time period, no visible aggregates were observed but the concentration of transparent exopolymeric particles (TEPs) increased from 14.4 to 547.8 mg eq Alginic Acid L⁻¹ indicating successful production of aggregate precursors and requirement of longer incubation periods for the production of visible aggregates with oceanic waters.

[Keywords: Transparent polymeric particles, marine aggregates, plankton wheel, roller table].

Marine snow or marine aggregates lead to transport of organic matter into the deep sea¹. Several marine organisms including prokaryotes, microalgae and macroalgae release copious amounts of dissolved organic carbon in the form of extracellular polymeric substances (EPS)^{2,3,4}. Mucilaginous carbohydrates surrounding the cells of flagellates also contribute to EPS. Heterotrophic bacterial processes on the biologically labile organic carbon of the EPS gradually convert them into increasingly refractory material, which then coagulate to form fibrillar, sticky mucopolysaccharides. These are light and transparent in nature and are therefore called “transparent exopolymeric particles”^{5,6}. TEPs collide against each other randomly and by virtue of their stickiness, they clump together to form aggregates. The weight of these aggregates facilitates their sinking in the deeper waters. Larger the size of the aggregate, faster is its sinking rate⁷.

Marine aggregates have been widely studied in recent years. Researchers have been collecting them by scuba diving^{8,9}. The process for collection of aggregates from natural environment is important as during the process it is necessary to retain the structural integrity of an aggregate. Though

turbulence facilitates colloidal fibrils and TEPs to coagulate, resulting in formation of larger aggregates^{9,10}, they are equally prone to tear apart due to that¹¹. Hence, *in vitro* generation of aggregates, rather than their collection from water column, would facilitate their studies. They have been successfully generated in laboratory by means of various instruments and through the agency of various sources like bacteria, diatoms, macroalgal detritus, organic matter derived from seaweeds and benthic suspension feeders¹²⁻²¹ (Table 1). Devices like rolling tables (plankton wheels) and Couette flocculators have been used for production of aggregates in laboratory²²⁻²⁹.

A few precautions need to be taken to produce aggregates in laboratory conditions using these instruments. Firstly, if they are to be produced from natural waters, then physical conditions more or less simulating those present in natural waters need to be maintained. Secondly, there should be provision for periodical sampling during the experiment. Thirdly, generation of aggregates in oceanic waters requires setting up the instrument on board a ship. The original plankton wheel designed by Shanks & Edmondson (1989) used gravity to keep bottles on rotating drive shaft²². As a ship is subjected to continuous rolling and pitching movements, their design which requires firm and steady base is not advantageous for use on board the ship. Robins & Bellan (1986) devised “IMER plankton wheel” for use on board a ship²⁷.

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Table 1—List of various sources from the literature used for the production of TEPs and aggregates in laboratory

Source	References
Bacteria	Biddanda 1985 ¹²
Diatoms / their extracellular products	Prieto <i>et al.</i> 2001 ¹³ , Unanue <i>et al.</i> 1998 ¹⁴ , Mari 1999 ¹⁵
EPS of bacteria and diatoms	Bhaskar <i>et al.</i> 2005 ¹⁶
Macroalgal detritus, exudates	Thornton 2004 ¹⁷ , Ramaiah <i>et al.</i> 2001 ¹⁸
Organic matter derived from sea weeds	Biddanda 1985 ¹²
Organic matter derived from benthic suspension feeders	McKee <i>et al.</i> 2005 ¹⁹ , Heinonen <i>et al.</i> 2007 ²⁰
Seawater	Radice <i>et al.</i> 2006 ²¹

It has various advantageous like use of differently sized experimental bottles, variable speed control for rotation of experimental bottles. The design is sophisticated and bulky. Crocker & Gotschalk (1997) designed a plankton wheel which could be used on board but this requires a continuous flow of water for the rotation of bottles²⁸. Continuous flow of water can be maintained by constant pumping which is expensive in terms of consumption of electricity. Kim & Kang (2003) devised a bubble-powered plankton wheel for grazing experiments over long periods²⁹. Though it is specially manufactured for use on board, periodical sampling from the same is difficult as the experimental bottles are housed within a cylindrical container that is placed in seawater and rotated with the help of bubbles generated in the seawater. It is thus useful only for experiments with long incubation periods that need no periodical sampling in between.

Instrument design—We have devised a plankton wheel or roller table which is simple in design, easy to handle, cost-effective and allowing easy periodical sampling from selective experimental bottles, whenever required, without much disturbance to the rest. This design (**Fig. 1**) consists of a 600 mm long cage made up of 4 aluminium rods of diameter 12.5 mm mounted horizontally on 2 PVC end flanges of diameter 140 mm and 20 mm thick, in which the sample bottles are housed. The entire cage is mounted on a steel frame with the help of pillow block bearings (P208), which are coupled to the end flanges by 10 mm × 100 mm long aluminium shafts. An electric motor is bolted on one end of the frame which drives the cage using a pulley and belt arrangement. A flange is also provided in the centre of the cage to firmly secure the bottles and minimize sagging. A single phase 230 V, 50 Hz, 0.1 HP reversible synchronous motor is used which has a fixed rpm of 60. A reduction of 1:3 in speed is achieved with the help of small pulley of 25 mm diameter coupled to the motor shaft and a big pulley of 75 mm diameter coupled to the shaft connected

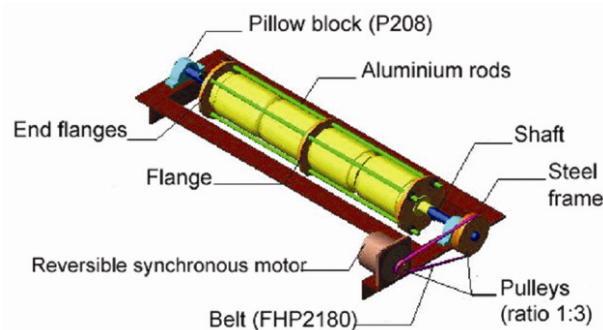


Fig. 1—Diagrammatic illustration of the plankton wheel designed in the present study.

to the pillow block and driven by a belt (FHP2180). The final speed of rotation of 23 rounds per minute is achieved.

The sample bottles of predetermined size holding around 1 L of water are laid in the cage in horizontal manner and the end flanges are tightened using nuts. Care must be taken to prevent over tightening. The cage is then mounted on the shaft connected to pillow block. We used Sunpet bottles with screw capped lids that were surface sterilized with ethanol prior to use.

Experiment in the laboratory (with coastal water)—A preliminary experiment to check the efficiency of our plankton wheel for production of aggregates was carried out in laboratory on land. Surface seawater collected from Dona Paula Bay was filled in the bottles and incubated on the plankton wheel at a constant speed of rotation of 23 rpm under diffused light conditions and controlled room temperature of around 30°C [similar to sea surface temperature (SST)] for 11 days. Hundred mL sample was withdrawn from the bottles every day till day 4 and then on day 6, 9 and 11. The samples were fixed with formalin up to a final concentration of 2% and stored under refrigeration until further analysis. The size of aggregates was measured under microscope within two days of fixation. The amount of sample withdrawn was replaced with water sampled fresh from Dona Paula Bay and filtered

through 0.22 μm . Aggregates were generated in the bottles (Fig. 2) and they varied in size from 0.71 to 11.3 mm^2 (Fig. 3). The average size of aggregates in Dona Paula waters was also measured at the time of sampling and found to be 0.6 mm^2 .

Experiment on board (with oceanic water)—After the successful generation of aggregates on land, an experiment was carried out on a cruise to equatorial Indian Ocean on board ORV Sagar Kanya (SK 228) during September 2006. Surface seawater was collected at 0° N 80.5° E using SeaBird CTD. The seawater collected through CTD was filtered immediately through 20 μm mesh, filled in the bottles and incubated on the plankton wheel at a constant speed of rotation of 23 rpm under diffused light conditions and controlled room temperature of around 25°C [similar to sea surface temperature (SST)] for 15 days. Fifty mL samples were withdrawn from the bottles every 3-4 days and fixed with formalin up to a final concentration of 2% and stored under refrigeration. No visible aggregates as that seen in the coastal water experiment were formed in these bottles. This could be due to the presence of different TEP precursors¹⁹ since small particles (0.6 mm^2) that were present in the coastal waters during the time of sampling, were not observed in the oceanic water. Incubation for longer time period could have probably led to the formation of aggregates but the experiment could not be continued for long time due to time constraints and ship availability. Hence, TEPs in these water samples were enumerated by Alcian Blue staining method of Passow and Alldredge (1995). This was done by filtering 10-25 ml of water sample through 0.4 μm white polycarbonate filter papers³⁰. The amount of water withdrawn from the bottles for the analysis was replaced with 0.22 μm filtered seawater collected from the same location and stored at 25°C.

TEPs varied from 157.8 to 547.8 mg eq AA L^{-1} in the experimental bottles (Fig. 4). TEPs concentration decreased sharply from day 3 to day 6 followed by a rise on day 10 that remained almost constant till day 15. The concentration of TEPs in bottles incubated on the plankton wheel was more than that found in the natural sample collected (14.4 mg eq AA L^{-1}). Higher concentration of TEPs in plankton wheel bottles than in the natural waters indicated that the plankton wheel facilitated the production of TEPs.

Present plankton wheel design has several advantages over all the previously known designs in

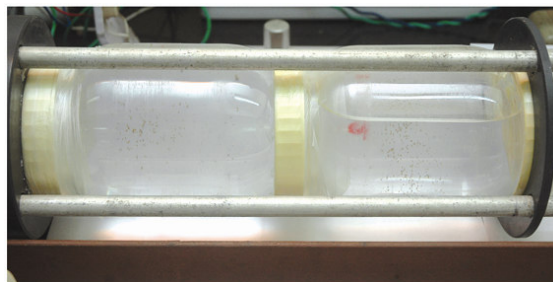


Fig. 2—Photograph of bottles mounted on plankton wheel containing aggregates generated from coastal waters during an experiment on-land.

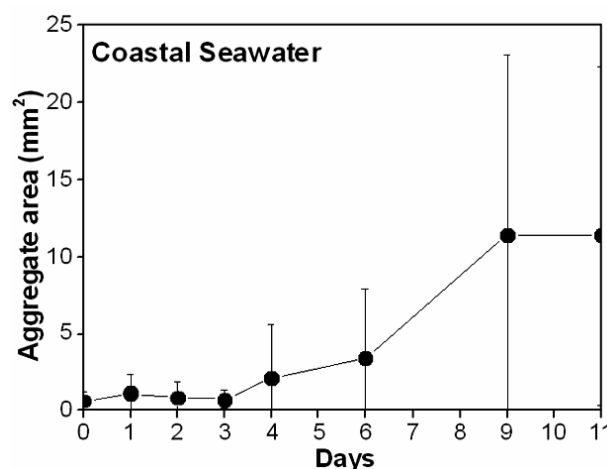


Fig. 3—Distribution of aggregate size in terms of area (mm^2) with respect to time, aggregates being generated from coastal waters of Dona Paula Bay in the plankton wheel.

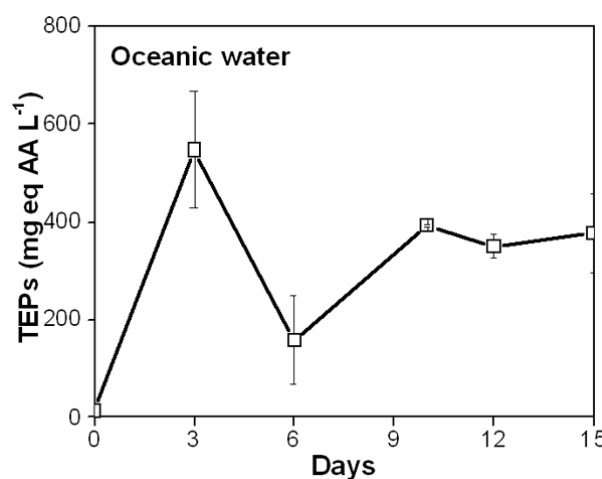


Fig. 4—Distribution of TEPs in plankton wheel bottles with respect to time generated from equatorial Indian Ocean water on board ORV Sagar Kanya (SK 228).

that it can be easily dismantled, transported and assembled with ease using very few tools. The device is quite stable and is not affected by rolling-pitching movements of the ship. Hence, it can also be used to carry out experiments on-board as was used in the experiment described in this paper. The cost of the set up is minimal. It does not require any continuous flow of water as in case of seawater-powered plankton wheel, thus making its design simpler. Since the electric motor is properly cased and bolted at one end of the frame, there is minimal risk of electric shock. The design is handy and not inconveniently large and weighs only 17 kgs. Thus, this is a very convenient tool for production of marine aggregates.

One limitation of this design is that only bottles of predetermined size can be used. Fixing of different size bottles will require a modification in the size of the cage unlike IMER plankton wheel. A constant speed of rotation can be maintained. Speed can be varied if desired by the use of variable speed electric motor instead of fixed speed motor used in this study. The speed can also be varied by suitably choosing the pulleys on the electric motor shaft and on the pillow block shaft.

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