Stimulated bioluminescence by fluid shear stress associated with pipe flow

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Abstract: Dinoflagellate can be stimulated bioluminescence by hydrodynamic agitation. Two typical dinoflagellate (Lingulodinium polyedrum and Pyrocystis noctiluca) was choosed to research stimulated bioluminescence. The bioluminescence intensity and shear stress intensity were measured using fully developed pipe flow. There is shear stress threshold to agitate organism bioluminescence. From these experiment, the response thresholds of the stimulated bioluminescence always occurred in laminar flows at a shear stress level of 0.6-3 dyn/cm². At the same time, the spectral characteristic of dinoflagellate was recorded, the wavelength of them is about 470nm, and the full width at half maximum is approximate 30nm.

1. Introduction
Bioluminescence is a ubiquitous and conspicuous nighttime phenomena in the sea waters. Bioluminescent plankton can be stimulated by flow fields associated with ship wakes[1], breaking waves[2], swimming dolphins[3], small fish, divers, and swells moving past submerged objects. Marine bioluminescent organism occur at all accessible depths and domains, often in immense numbers[4]. And the typical organisms responsible for this displays are primarily dinoflagellates, unicellular marine plankton which are the most abundant sources of bioluminescence in near-surface, coastal waters. Because they can be cultured, dinoflagellates are suitable for laboratory study using well-characterized flow fields. It has been suggested that concentrations of dinoflagellates as low as 100 cells per liter are sufficient for bioluminescence at night to highlight moving objects[5]. Therefore, the research of stimulated bioluminescence can have many application, such as, flow visualization technology, object detection and identification in water at night in the future.

It is a common experience that luminous marine organisms can be mechanically stimulated to produce bioluminescence. Ship’s wakes and breaking waves are often common source of observable bioluminescence. However, in terms of quantifiable hydrodynamic parameters, the conditions related to bioluminescent excitation have been poorly characterized. It was thought that investigating the response of bioluminescence in a well described flow field would provide some quantitative measure of the attendant state of mechanical agitation. Pipe flow was chosen as it is one of the most studied and best hydrodynamically characterized over a wide range of flow conditions[1]. A series of experiments were performed in the laboratory where the bioluminescence of seawater flowing through a glass pipe was measured at different flow rate. To detection stimulated bioluminescence of ocean organisms in the future, the spectral properties of 2 species of the dinoflagellates were also researched.
2. Pipe flow field stimulated system and theory

In order to compare the response of bioluminescence in different quantitative flow field, and to determine the threshold values of wall shear stress which are necessary to stimulated bioluminescences, the study of bioluminescence in pipe flow was employed. Pipe flow was chosen to stimulate bioluminescence because (1) it is well characterized by hydrodynamic method, (2) wall shear stress \( \tau \) can be easily calculated by the pressure drop, (3) it offers a wide range of laminar and turbulent stimuli, (4) and new organisms are constantly entering the flow field. In fully developed laminar flow there is a parabolic velocity profile which results in a linear gradient of shear. The highest magnitude of shear is at the wall of the pipe; shear decreases to zero at the centerline.

2.1. The theory of fully developed pipe flow

Pipe flow is considered fully developed when its velocity profile is no longer evolving downstream. With no mean acceleration, the pressure drop, \( \frac{dP}{dx} \), across the face of any concentric, cylindrical control volume of radius \( r \) must be balanced by the shear stress acting on its perimeter. This relationship can be expressed as

\[
\tau(r) = -\frac{dp}{dx} \cdot \frac{r}{2}
\]  

with at the pipe wall, \( R \), becomes

\[
\tau_{wall} = -\frac{dp}{dx} \cdot \frac{R}{2}
\]

then the relationship of the shear stress in flow field and the wall shear stress can be reduced to

\[
\tau(r) = \tau_{wall} \cdot \frac{r}{R}
\].

The formula expresses a general law of the linear stress distribution, regardless of whether the flow is laminar or turbulent. In order to determine if the pipe flow was fully developed, laminar, turbulent, or transitional, the Darcy friction factor\(^{[1]}\):

\[
\lambda = -\frac{dp}{dx} \cdot \frac{D}{\frac{1}{2} \rho U_{avg}^2}
\]

and the Reynolds number is defined as,

\[
Re = \rho \cdot \frac{U_{avg} D}{\mu}
\]

where \( U_{avg} \) is the average flow speed, \( \rho \) is the fluid density, \( \mu \) is the dynamical viscosity, \( D \) is the pipe diameter.

When the pipe flow is in the laminar flow, the sole source of shear stress in laminar flow is due to viscosity. The viscous stress developed in laminar flows of most common fluids are found to be equal to the product of the kinematic viscosity and the local velocity gradient. Fluids adhering to the linearity between shear stress and velocity gradient are referred to as Newtonian. In fully developed laminar pipe flow of a Newtonian fluid the following relationship holds:

\[
\tau(r)_{lam,ir} = \mu \cdot \frac{du}{dr}
\]

where \( u(r) \) is the velocity along the pipe axis, measured at radius \( r \) from the pipe centerline. In fully developed laminar pipe flow, the theoretical relation\(^{[6]}\) between \( \lambda \) and \( Re \) is

\[
\lambda = \frac{64}{Re}
\]

which can also be expressed, via equations (2), (3), and (6), as

\[
\tau_{wall} = \frac{8 \rho U_{avg}^2}{Re}
\]

According to equation (5), equation (7) can be described as
When the pipe flow is in the turbulent flow, the empirical relation by Blasius\(^5\) is
\[
\lambda = 0.3164/\text{Re}^{1/4}
\]

The same way as before, the wall shear stress in turbulent can be expressed as
\[
\tau_{\text{wall}} = 0.04\rho U_{\text{avg}}^2/\text{Re}^{1/4} = 0.04(\mu \rho / D)^{1/4} U_{\text{avg}}^{7/4}
\]

From these equations, it is important to realize that \(\tau_{\text{wall}}\) increase with the flow speed in both laminar and turbulent flow. Therefore, in the experiment, the wall shear stress can be changed with the pipe flow speed.

2.2. Bioluminescence experimental apparatus

2.2.1. Flow-field measurements

Cultures were exposed to fully developed pipe flow as showed in Figure 1. The experimental apparatus consisted of a 50 liter transparent-plastic aquarium, arranged to discharge seawater vertically downward into a gently contraction, then through a 6.5mm internal diameter clear polycarbonate glass pipe 1 meter in length. The contraction was carefully designed using a cubic equation to give the smallest boundary layer thickness at the entrance to the test pipe. Volume flow rate, measured at the discharge with a rising-ball flow meter, was divided by the pipe area to obtain the average water velocity. Flow field through the pipe was manually controlled by a valve. The mean velocity (\(U_{\text{avg}}\)) of each steady flow was determined by weighing the amount of water collected over a measured time and dividing by the pipe area. The pipe was equipped with pressure taps, the pressure drop along the pipe was measured with a variable reluctance differential transducer. The relationship between pressure drop and flow rate was used to characterize if the flow was fully developed and laminar, turbulent, or transitional.

2.2.2. Experimental organism

\textit{Lingulodinium polyedrum} and \textit{Pyrocystis noctiluca} are the common oceanic species of dinoflagellate. They were grown in f/2 medium minus silicate and maintained in a culture chamber at 20±2°C on 12:12 light:dark cycle. Day illumination was provided by cool-white fluorescent tubes at approximately 10w/m². Subjective day always stared at 17:00 and subjective night at 05:00. Experiments were always conducted between 07:30 and 11:30, so the cells were in the middle of the dark phase. Cell abundance was determined by counting samples under an optic microscope. The images of live cells of the bioluminescent dinoflagellates are displayed in Figure 2. Cultures used for the experiments were inoculated every 4 weeks. And the concentrations which was determined by
counting samples under a microscope used for the experiments were 28 -500 cell/ml or as otherwise stated. At the beginning of the darkphase, the pipe flow apparatus fullled with organisms was covered with opaque sheeting. When the organism bioluminescence is maximal, the test commenced in the middle dark phase.

2.2.3. The bioluminescen ce measurements

Bioluminescence was measured by photomultiplier and its property was analysed by spectrometer. A photomultiplier tube located 65cm (100 pipe diameters) from the pipe inlet was used in photon count mode to measure the light flux emitted by the dinoflagellates. The photon number was integrated over a chosen period of 100ms. The sensitive area diameter of PMT is 25mm, and the dark current is equal to less than 100 counts/s at 25°C. The PMT is sensitive enough to detect single flashes. The exported pulses of PMT was counted by a counter controlled by a computer through software. At the same time, the optical properties of bioluminescence were recorded by spectrometer.

3. The experimental data and analysis

3.1. The relationship between bioluminescence and wall shear stress

According to hydrodynamics the relation between Darcy friction factor and Reynolds number indicates whether flow is laminar, turbulent, fully developed, or transitional. Calculations of mean velocity and wall shear stress confirmed that the cells in fully developed flow when the measurements of bioluminescence were made (Figure 3). As showed in figure, the empirical data is consistent with the theoretical value. So the measure method of flow field is feasible.

![Figure 3](image1.png)  
**Figure 3.** Relationship between wall shear stress and mean velocity for pipe flow. The solid line is the theoretical relationship for laminar flow, symbols represent empirical values.

When cultures of dinoflagellate were subjected to wall shear stress of pipe flow, the response pattern was characterized by an excitation threshold as showed in figure 4. Culture response varied with flow conditions. Flows with a wall shear stress below or near the threshold value, luminescence was similar to background levels. As the flow rate and thus the wall shear stress increased, so did the rate of flashes and bioluminescence intensity of individual flashes. From the figure, the threshold of the *L. polyedrum* is presetned about 3 dyn/cm², and more than that of the *P. noctiluca* about 0.6 dyn/cm². The line represents the least-square power law regression between wall shear stress values.

![Figure 4](image2.png)  
**Figure 4.** Mean bioluminescence intensity as a function of wall shear stress.
and mean tensity. For L. polyedrum, the stresses within the range of 1-10dyn/cm2 light emission was proportional to the 2.8 power of shear stress (R²=0.94). And to P. noctiluca the slope of line was about 2.6 (R²=0.97).

3.2. The spectral properties of bioluminescence

Measurement of bioluminescence spectral properties are complicated by the frequently dim and transient nature of marine organisms luminescence. The dinoflagellate spectra are confine to the range of 460-490nm as showned in figure 5. In this experiment, the maximum wavelength of L. polyedrum is approximate 474nm, full width at half maximum (FWHM) is about 30nm; and for P. noctiluca, λmax=471nm, FWHM=32nm.

![Figure 5. The spectral characteristics of dinoflagellate. The cell concentration of L. polyedrum is 463 cells ml⁻¹; that of the P. noctiluca is about 28 cells ml⁻¹.](image)

4. Conclusions

According to previous reporter, bioluminescence intensity will depend on the species abundance and concentration of luminescent organisms present, as well as their agitation, light and nutrient prehistory[3]. The present experiment shows that pipe flow as the source to stimulate organism bioluminescence is feasible. And the study also present that dinoflagellate such as L. polyedrum, P. noctiluca was stimulated bioluminescence when they responded to well-characterized hydrodynamic forces. The response of dinoflagellate to hydrodynamic stimulation was characterized by wall shear stress, and at the same values of wall shear stress, the response was similar for laminar and turbulent flows.

The thresholds of wall shear stress that stimulated bioluminescence occurred depending on dinoflagellate species. To some extent, the stronger of the wall shear stress became, the more mean bioluminescence intensity produced by dinoflagellate. On the basis of these properties, the study of ship wake bioluminescence and flow visualization technology can be developed, furthermore these technology can have many applications, for examples, controlling and guiding torpedoes.

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