Laser Innovations for Research and Applications

journal homepage: https://lira.journals.ekb.eg/

ISSN: Print 3009-6359 / Online 3009-6472

Monitoring pathogenic bacteria in water using Femtosecond laser

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Abstract

Water contamination represents a significant threat to both ecological sustainability and human life. 80% of the global population suffers from severe water pollution. Different types of pollutants adversely affect water resources. The most significant health risks associated with drinking water in developing countries are caused by pathogens, which are microorganisms causing diseases including bacteria, viruses, and parasites that are transmitted via the oral-fecal pathway. Most water-borne diseases result from microbial pollution, according to the WHO's drinking water quality guidelines, fourth edition. It is essential to diagnose these pathogens rapidly to impede the spread of the corresponding diseases. This review presented the most common bacterial detection techniques, microbiological culturing method, enzyme-linked immunosorbent assay (ELISA), and polymerase chain reaction (PCR) technique, and discussed the laser induced fluorescence (LIF) spectroscopy technique as a potential method for bacterial detection, which is a sensitive, accurate, and dependable method for quickly and immediately identifying harmful bacteria.

Keywords: Laser-induced fluorescence (LIF), Femtosecond laser, Pathogenic bacteria, *E. Coli*, *Enterococcus faecalis.*

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1. Introduction

1.1. Water pollution

Water scarcity exists in many areas worldwide, with over one billion people missing the accessibility of clean water (Kılıç, 2020). According to some recent estimates, about 900 million people in the world are without access to safe drinking water and about 2.6 billion people are without improved sanitation facilities (Nations, 2012; Organization, 1950). Water contamination is a key factor in the water crisis (Dwivedi, 2017). Water pollution refers to any change in the physical, chemical, or biological characteristics of water that has a negative impact on the health of all living beings (DeZuane, 1997). The term "water pollution" refers to the procedure resulting in water being unsafe for consumption due to the introduction of excessive levels of harmful substances (Olaniran, 1995). 80% of the global population suffers from severe water pollution (Owa, 2013).

Different types of pollution adversely affect water sources (Chaudhry & Malik, 2017), such as the discharge of heated water from geothermal or nuclear power plants and industries where water is used as a coolant results in rising water temperature, which is a form of physical pollution. As the temperature rises, the dissolved oxygen in the water reduces which adversely affects aquatic life. Furthermore, in case organic materials are present in water, the multiplication of bacteria and depletion of the dissolved oxygen will occur (Davidson & Bradshaw, 1967). Dye, cosmetic, and petroleum waste are significant examples of organic contaminants that constitute substantial hazards to animals, plants, and humans and cause severe burns, vomiting, epileptic attacks, and carcinogenic effects (Hanafi & Sapawe, 2020). Inorganic nitrogen compounds (nitrate, nitrite, and ammonium) and inorganic phosphates that result from the

release of agricultural, domestic, and industrial waste. Nitrite causes hazards to human health, such as stomach and liver diseases and esophageal cancer (Rekha Kathal et al., 2016). Radioactive materials may be released into water sources, where they are consumed by living things and passed on to people, resulting in different genetic mutations. The most significant radiation that results in bone cancer is radium. Additionally, a physiological alteration results from the accumulation of radioactive substances in water (Eisenbud & Paschoa, 1989; Hatch, 1953). Pathogenic bacteria, viruses, and parasites are examples of significant contaminants that cause biological pollution. The feces of both people and animals are the carriers of these contaminants. When they combine with wastewater or agricultural runoff water, they are transmitted to the water, resulting in many infectious diseases in humans, like cholera (Pandey et al., 2014). Low water quality is estimated to cause increasing mortality worldwide every year, with pathogenic bacteria thought to be responsible for approximately 26% of all mortality (Carr & Neary, 2008).

1.2. Pathogenic bacteria

Pathogens are microbes causing disease including viruses, bacteria, and parasites that are transmitted by the oral-fecal pathway and are the most significant health hazards related to drinking water in developing countries (Ashbolt, 2004; Gerba, 2009). Human feces and untreated sewage discharge into water are the two main sources of pathogens found in water resources (Naidoo & Olaniran, 2014). *Clostridium perfringens*, *Escherichia coli*, *Enterococcus faecalis*, and Coliforms bacteria are the most frequent pathogens in water (Some et al., 2021). The most typically investigated biomarkers determining the degree of fecal pollution are *E. Coli* and *Enterococcus. faecalis* (Hussain et al., 2007). Extended environmental survival—especially in the maritime environment—is a distinguishing characteristic of *Enterococci* over coliforms such as *E. coli* (Figueras et al., 2010). They are consequently two times as resistant to treatment as fecal coliform (Scully Jr et al., 1999). *Enterococcus faecalis* is the causative agent of up to 90% of human enterococcal infectious diseases (Kayaoglu & Ørstavik, 2004). *E. faecalis* is now one of the three most prevalent pathogens responsible for clinical infections in the aged and people with immune deficiencies, such as urinary system infections, bacteremia, and bacterial

endocarditis (Morrison & Wenzel, 1986; Murray, 2000). The primary agent of water-related infections like digestive tract infections is *E. Coli* (Ashbolt, 2004). A variety of diseases, such as dysentery, sepsis, urinary system infections, and diarrhea-associated consequences resulting in hemolytic-uremic disorder, can be induced by *Escherichia coli* (*E. coli*) (Pokharel et al., 2023). These illnesses still claim the lives of hundreds of millions of individuals each year all over the world (Croxen & Finlay, 2010; Donnenberg, 2002). Intestinal and urinary system infections are the most prevalent infectious diseases detected in healthcare settings that are typically caused by *E. coli* (Chakupurakal et al., 2010; Murugan et al., 2012). It is essential to diagnose these pathogens rapidly to impede the spread of the corresponding diseases (Hoorfar, 2011; Tängdén & Giske, 2015).

1.3. Most common bacterial detection techniques

The most prevalent techniques employed for bacterial identification are the microbiological culturing method, enzyme-linked immunosorbent assay (ELISA), and PCR technique (Shen et al., 2021; Wang et al., 2021).

I. Microbiological culturing technique

The bacterial culture method is a process in which the enrichment of a specific bacteria on specific mediums is used to separate it from the surrounding microorganisms (Kim & Kim, 2021). Numerous culture mediums are available, including liquid cultivation media and semi-solid agar plates (Bonnet et al., 2020). Agar-based multi-purpose media such as blood agar have been utilized in the cultivation procedure because they enable the enrichment of a variety of pathogens (Kim et al., 2022). It is crucial to employ a selective culture medium to identify specific bacteria (Rajapaksha et al., 2019). Since the selective cultural medium limits the number of circulating microorganisms that can proliferate, increasing the possibility of separating specific bacteria (Váradi et al., 2017). Species-level characterization for several prevalent and newly discovered diseases of samples from nature is unreliable by applying culture techniques and biochemical approaches (Dong et al., 2008). Conventional detection methods are unsuitable in the case of the intended bacteria being encased in biofilms. Pathogenic bacteria may pass into a viable but non-culturable phase after long-term contact with water, during which they maintain their infectious capability

but cannot be identified by culture (Cenciarini-Borde et al., 2009). For instance, several microorganisms, like Helicobacter pylori and Vibrio cholera, cannot be cultivated on prevalent culturing media as they can be found in food, water, or ecological samples in a stage known as "viable but not cultivable" (VBNC) (Law et al., 2015b; Ramírez-Castillo et al., 2015). Furthermore, bacterial concentrations could be sufficient to induce infection even when they are too low for identification by cultures (De Kievit & Iglewski, 2000). These procedures take a long time, it takes 18 to 72 hours to cultivate bacteria to a density that can be detected (Zeng et al., 2018). Genus- and species-level identification follows isolation, and commercially accessible kits of biochemical assays are commonly used for this purpose (Herbel et al., 2013).

II. Biochemical techniques

One of the most effective traditional techniques for identifying pathogenic bacteria is the biochemical procedure, which is typically carried out the following the culture procedure (Chauhan et al., 2017). Biochemical analyses mainly work on variations in bacterial biochemical reactions (Rodrigues et al., 2017). For example, the capacity to decompose starch (Hameed et al., 2018). Several drawbacks have been observed with this method (Granada, 2018). In particular, certain biochemical tests are insufficient for detecting specific bacteria, necessitating the use of a supplementary set of biochemical analyses (Ogunware et al., 2020). Another drawback has been investigated regarding the precision of the biochemical method. For instance, the latest research examining the antibacterial effect of dyes from soil bacteria indicated that *E. coli*, which is positive for methyl red and has even been employed as a traditional positive control for the methyl red test, is negative (Qayyum et al., 2020). It may be concluded that the biochemical method of detecting bacterial infections in nutrients, water, and ecosystems is ineffective and imprecise (Nnachi et al., 2022).

III. Immunoassay technique

According to the concept that antibodies and antigens bind selectively

immunological assays are employed as a bacterial detection method (Chang et al., 2016). The prevalent immunological methods for identifying pathogenic bacteria are the enzyme-linked immunosorbent assay (ELISA), immuno-magnetic separation (IMS) (Välimaa et al., 2015), and enzyme immunoassay (EIA) (Chapman et al., 1997; Qadri et al., 1990). Compared to a culture, they take a shorter time for the test (Mattingly et al., 1988).

A. Enzyme-linked immunosorbent assays (ELISA)

Using a specific antibody that has been attached to an enzyme when the antibody and antigens are linked, an unidentified bacterial antigen is identified via ELISA. A perceptible color variation will result from the correct attachment (Koivunen & Krogsrud, 2006). False-positive results could arise from the antibody's influence on the interaction and cross-reactivity, which could lead to low selectivity and precision. ELISA is frequently used in conjunction with additional identification methods to avoid this (Diaz-Amigo et al., 2010; Hayrapetyan et al., 2023). This method lacks the ability for real-time identification of bacteria (Alahi & Mukhopadhyay, 2017).

IV. PCR technique

One of the most widely used techniques for identifying bacteria is the polymerase chain reaction (PCR) (Fratamico, 2003; Oh et al., 2016). Each type of bacteria is characterized by its nucleic acid composition (Jensen et al., 1993; Naravaneni & Jamil, 2005). Nucleic acid (DNA or RNA) can be multiplied to produce billions of copies from a limited number of copies in a sample through PCR to assist pathogen detection and diagnosis (Bouchez et al., 2016). The polymerase enzyme is used in a sequence of heat-cycling procedures to accomplish this. High temperatures are used in the decomposing process of the double-stranded DNA molecules. Following that, the polymerase enzyme extends the primer-template complex after selected primers bind to complementary DNA strands. The amplified PCR product is observed as bands after being stained with ethidium bromide on an electrophoresis gel (Maurye et al., 2017). Quantitative PCR analysis determines the quantity of the DNA of a particular pathogen per mL of water (Brettar & Höfle, 2008). PCR technique is insensitive in case of a low concentration of pathogenic bacteria in water, so a

culture procedure must be performed for multiplication before starting the PCR procedure (Noble & Weisberg, 2005).

Common sales reagents for PCR techniques are the Takara Genomic DNA extraction kit (Liu et al., 2019), QI Amp DSP DNA mini kit (Allard et al., 2019), and KAPA HiFi Hot Start Ready-mix (Pereira et al., 2017). One of the drawbacks of the PCR technique is the costly prices of these reagents (Hønsvall & Robertson, 2017). This method is technologically and financially inappropriate for countries with restricted resources because it requires efficiently prepared labs and expert technicians (Nnachi et al., 2022). Furthermore, the precision of the PCR process may be affected by particular variables, such as the efficiency of enzymes and DNA template characteristics, that inhibit primer annealing to the DNA template (McDOWELL et al., 1999). PCR requires that the intended genetic code be known beforehand (Blankenship & Yayanos, 2005). For on-site testing, the PCR technique is unsuitable because of the complicated procedure that requires bacterial genome extraction and the utilization of a thermocycler (Ma et al., 2021; Saptalena et al., 2015).

Most conventional detection techniques for water quality use chemicals, however, using chemicals during testing may result in additional pollution to the ecosystem (Chen et al., 2022). Moreover, the highly costly chemicals used, the long time to obtain results, and the complicated operation methodology constitute significant drawbacks of the techniques mentioned above (Lazcka et al., 2007; Shih et al., 2015). The time-consuming conventional detection methods of pathogens may result in fatal harm to the patients undergoing medication because they are at risk of bacterial infections resistant to antibiotics (Giana et al., 2003). As a result of these limitations, a rapid method of diagnosis of pathogens is highly needed to preserve public health (Law et al., 2015a; Ramírez-Castillo et al., 2015). Intrinsic fluorescence spectroscopy is highly recommended as a rapid technique for bacterial detection throughout numerous samples because it is an easy, reliable, and affordable way to identify pathogens without the need for labelling (M. S. Ammor, 2007; Shelly et al., 1980). Laser-induced fluorescence spectroscopy (LIF) has been widely used to monitor water quality (Du et al., 2022b; McLellan et al., 2012).

V. Laser-induced fluorescence spectroscopy technique

When atoms absorb photon energy, they undergo a transition into a certain excited energy state, then they de-excite and return to the ground state via multiple pathways, among them fluorescence emission (Telle et al., 2007). Therefore, various mechanisms and phenomena that occur during the de-excitation process need to be studied to understand how matter interacts with light radiation.

A. Luminescence phenomena

The light emission known as luminescence occurs when incident photons are absorbed by matter and released as light photons; the emission wavelength is specific to the matter (Ashraf et al.).

Types of Luminescence:

Based on the energy source, luminescence is categorized into photoluminescence, electroluminescence, chemiluminescence, and bioluminescence (Murthy, 2015). The phenomenon of photoluminescence results from light photon absorption (Shinde et al., 2012). There are two pathways of photoluminescence: fluorescence and phosphorescence based on the type of excited state and lifetime (Valeur & Berberan-Santos, 2011).

B. Decay Process of Excited States:

A Jablonski diagram is mainly employed for explaining the mechanisms that occur throughout both light absorption and emission process (Jabłoński, 1935), as shown in Figure 1 (Bose et al., 2018b). It also demonstrates the transition probabilities that take place in excited states (Douglas et al., 2013). The singlet states (S) and triplet states (T) represent the electronic states of most matters (Itoh, 2012). Various vibrational energy levels exist in these electronic energy states (denoted by 0, 1, 2, etc.) (Möller & Denicola, 2002). The transition probabilities and the excitation energy determine the possibility of the molecule existing in one of the singlet excited states, Sn (Cartwright, 1978). Transitions are allowed from singlet excited states, where the excited orbital electron is coupled (on opposing spin) with the second orbital electron of the ground state,

to the ground state (Yang et al., 2016). Transitions are not allowed from triplet excited states, where the excited orbital electron shares the same spin direction as the ground-state electron, to the ground-state (Lower & El-Sayed, 1966). Multiple mechanisms often take place upon light absorption (Berera et al., 2009).

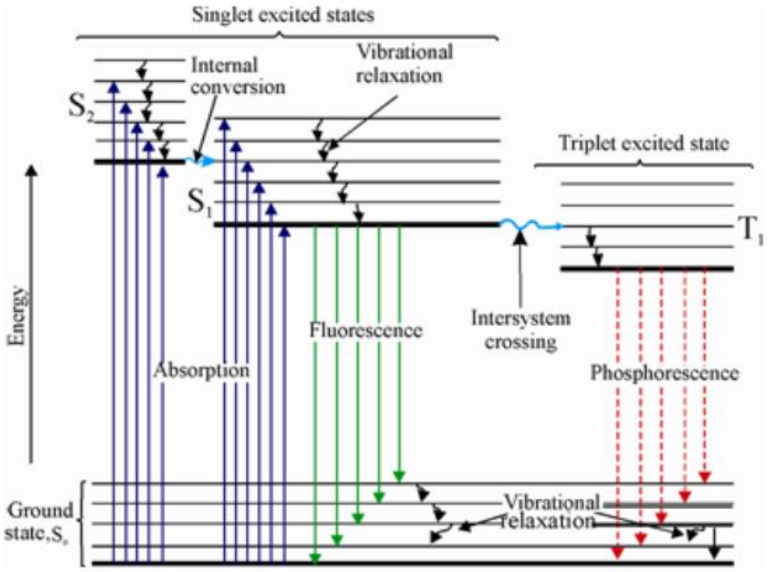

Fig. 1. A Schematic illustration of the Jablonski diagram. (Bose et al., 2018b)

1. Fluorescence

When molecules are excited to a higher excited singlet state (Sn), they decay to the higher vibrational level of the lower excited singlet state through a process called internal conversion (Zigmantas et al., 2001). Vibrational relaxation causes molecules at a higher vibrational level to rapidly descend to the lowest vibrational level of this state by transferring energy to other atoms through collisions (Rich & Treanor, 1970). Internal conversion is a non-radiative decay to lower excited energy that occurs between two electronic states with identical multiplicity, for instance, triplet-to-triplet or singlet-to-singlet states (Kalyani et al., 2017). When two electronic energy states are sufficiently close to cross-connect their vibrational energy levels, as is the case between S_1 and S_2, the internal conversion is easier to accomplish (Macpherson & Gillbro, 1998). Internal conversion typically occurs before emission because the fluorescence lifetime is 10^{-8} seconds (Noomnarm & Clegg, 2009). The emission of

fluorescence typically results from an excited state that has reached thermal equilibrium, the lowest vibrational level of S_1, to a higher vibrational level of the ground state, followed by fast decay to the lowest vibrational level of the ground state in 10^{-12} seconds (Knox, 1999).

2. Phosphorescence

When the vibrational levels of two states of excitation (T_1, S_1) intersect, electrons in the S_1 state change their spin orientation as in the first triplet state T_1 (Samanta et al., 2017). This process is termed intersystem crossing (Chuang et al., 1987). Phosphorescence is the emission from the triplet state T_1 to the singlet ground state, which is typically altered to longer wavelengths (lower energy) than fluorescence (Fleischauer & Fleischauer, 1970). Phosphorescence rates are many times lower than fluorescence because the transition from T_1 to the singlet ground state is very restricted (Crosby & Demas, 1970). Typically, the emission spectrum is used to represent data from the fluorescence spectrum (Warner et al., 1977). It represents fluorescence intensity vs wavelength (in nm) or wavenumber (in cm^{-1}) (Jameson et al., 2003).

C. Characteristics of the fluorescence emission spectrum

1. Stock shift

Emission energy is less than absorption energy as demonstrated in the Jablonski diagram (Lichtman & Conchello, 2005). Consequently, fluorescence predominantly shifted to longer wavelengths (Mukherjee & Chattopadhyay, 1995), as shown in Figure 2 (Mazi, 2019).

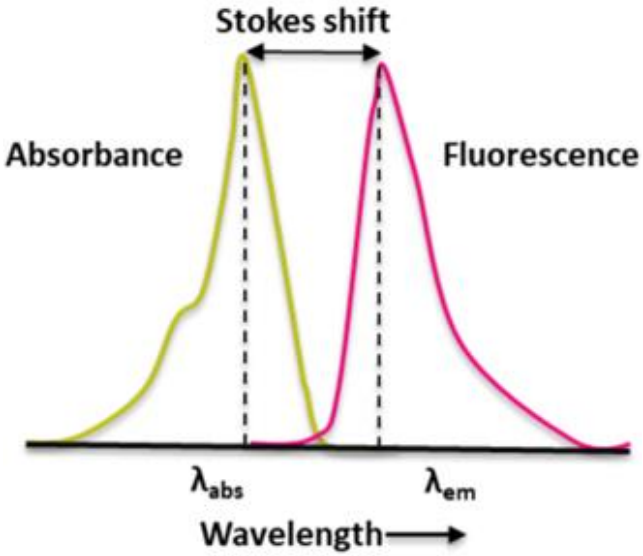

Fig. 2. A representation diagram of Stokes's shift. (Mazi, 2019)

This principle is known as the "Stokes shift" was first demonstrated in Cambridge in 1852 by Professor G. G. Stokes (Stokes, 1854).

2. Emission Spectra is independent of the excitation wavelength

Kasha's rule states that the fluorescence emission spectrum is independent of the wavelength of excitation (Kasha, 1950). The fluorophore decays to the lowest vibrational level of S_1 after the extra energy is rapidly lost following excitation into higher energy states (Bogdanov, 2002). Due to the quick relaxation that takes place in approximately 10^{-12} seconds, the emission spectra are typically unrelated to the excitation wavelength (Cushing et al., 2014).

3. Mirror image rule

This rule states that the emission spectrum of a fluorophore is frequently a mirror image of its absorption spectrum, especially when associated with the S_0 to S_1 transition (Sassara et al., 1997). The equivalent transitions contributing to both

absorption and emission processes are the cause of this phenomenon (Meredith et al., 2006). Moreover, the ground and excited state vibrational energy levels are spaced identically. Consequently, there is a similarity in the vibrational structures observed in the emission and absorption spectra (Laane, 1999).

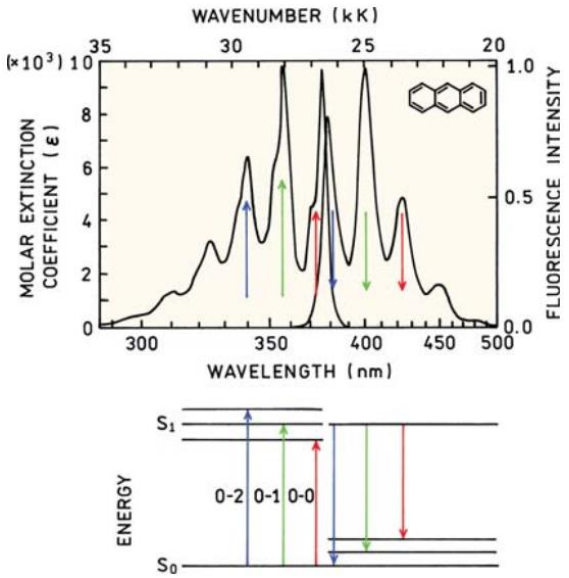

Fig.3. A diagram illustrates the Mirror-image rule through anthracene absorption and emission spectra. The numbers 0, 1, and 2 refer to vibrational energy levels. (Laane, 1999)

D. Factors Affecting Fluorescence

1. The nature of substituent groups

The fluorescence intensity is enhanced by molecules with electron-donating functional groups such as hydroxyl and amino groups (Zhao et al., 2021), weakened by molecules with electron-withdrawing functional groups such as Nitro, and carboxylic (Ansi et al., 2020), and uninfluenced by molecules with other groups, such as So_3H or NH^{4+} (Xiao et al., 2022).

2. Effect of temperature

While certain temperature-related procedures taking place in a matter may alter the fluorescence intensity, the fluorescence lifetime is typically not affected by temperature (Paviolo et al., 2013). An explanation for the drop in the fluorescence intensity with higher temperatures is the rising rate at which

electronic energy transforms into vibrational energy (internal conversion) (Andersson et al., 1995). The alternative theory is the intersystem crossing process or transition from a singlet excited state to a nearby nonfluorescent triplet state (Patil et al., 2013).

3. Quenching process

Quenching is the term describing any process causing the loss in the fluorescence intensity of a sample, and quenchers are compounds that may cause this loss (Green et al., 1992). Molecular oxygen, which inhibits nearly all recognized fluorophores, is one of the widely existing quenchers (Ware, 1962; Wilkinson, 1997). Halogens, amines, and acrylamide are also significant quenchers (Davis, 1973). The Stern-Volmer equation, equation 1 can illustrate the fluorescence quenching (Lakowicz, 2006):

$I_o/I = 1 + K_q \cdot \tau_F \cdot [Q]$ Eq. [1]

Where:

I_o: the intensity without a quencher

I: the intensity with a quencher

K_q: the quencher constant

τ_F: the fluorescence lifetime

Q: the quencher's concentration

4. Solvent effect

The solvent utilized in the fluorophore solution can have an impact on fluorescence spectra (Diwu et al., 1997). The variation of the position, intensity, and broadness of the fluorescence emission band are all indications of the solvent influence (Gemeda, 2017). The Fluorescence spectrum tends to alter to longer wavelengths (known as "red shift") as the solvent polarity is raised (Zhang et al., 2017).

5. Fluorophore concentration

There is a direct relationship between the concentration of the fluorophore and the fluorescence intensity (Bose et al., 2018a), according to the equation

that follows, equation 2 (Zacharioudaki et al., 2022).

$I_F = k \cdot I_o \cdot \Phi \cdot (Ɛ \cdot b \cdot C)$ Eq. [2]

Where:

I_F: Fluorescence intensity

k: a constant related to the instrument
I_o: the incident radiation
Φ: the quantum yield
Ɛ: the molar absorptivity
b: the path length
C: the concentration of a molecule

E. Fluorophores

In contrast to chromophores, which only absorb light, fluorophores absorb light and then radiate it back (Blohm et al., 2020; Pittalis et al., 2012). There are two main categories of fluorophores: intrinsic and extrinsic (Sahoo, 2011). Fluorophores introduced to a material to enhance its fluorescence emission spectrum are termed extrinsic fluorophores (Gonçalves, 2009). The fluorophores naturally found in cells and tissues are known as intrinsic fluorophores (Talamond et al., 2015). Nucleic acids, aromatic amino acids, and coenzymes are examples of intrinsic fluorophores that exhibit signature fluorescent characteristics and can be used as biologic indicators (Dunlap, 2008). Coenzymes and aromatic amino acids, like tryptophan, tyrosine, and phenylalanine, constitute the majority of bacterial intrinsic fluorophores (M. S. J. J. o. f. Ammor, 2007). Since aromatic amino acids make up 1% to 5% of the dry weight of a bacterium, they can be applied as biosensors to identify the kind, quantity, and class of bacteria (Pan & Transfer, 2015). Microorganisms differ in the types and amounts of lipids, coenzymes, and amino acids, which cause variations in the fluorescence emission spectra that can be used to distinguish between various bacterial species (Du et al., 2022b). Quantum yield, fluorescence lifetime, extinction coefficient, the maximum wavelength of absorption and emission, and their bandwidths (full width at half maximum)

constitute the significant optical characteristics of fluorophores (Joung et al., 2020).

F. Characteristics of fluorophores

The fluorophore quantum yield and fluorescence lifetime are the two most critical features (Zhang et al., 2014).

1. Quantum yield

It's represented as the ratio of photons emitted to photons absorbed (Crosby & Demas, 1971).

As shown by this equation, equation 3 (Lakowicz & Lakowicz, 1999):

$$Q = \frac{\Gamma}{\Gamma + k_{nr}} \qquad \text{Eq. [3]}$$

Q is the quantum yield, Γ is the number of emitted photons and k_{nr} is the nonradiative decay rate.

Any decay that doesn't induce photon emission is termed nonradiative decay (Schuurmans & Van Dijk, 1984). Such as transferring excess energy from an excited state to water's rotational and vibrational modes (Prezhdo & Rossky, 1996). If the rate of nonradiative decay is significantly lower than the rate of radiative decay, the quantum yield may approach unity, $k_{nr} << \Gamma$ (Hanifi et al., 2019).

2. Lifetime

It is known as the median duration time the fluorophore stays in the excited state before its decay to the ground state (Douglas, 1966). Since the fluorescence emission takes place quickly, the fluorescence lifetime is approximately a few nanoseconds (Clegg et al., 2003). The measured lifetime is the inverse of the overall decay rate, as shown in equation 4.1 (Alcala et al., 1987). The intrinsic lifetime is the fluorophore's lifetime regardless of any nonradiative decay and is determined by equation 4.2 (Beechem & Brand, 1985). Fluorophores in a sample can be distinct, utilizing the average lifetime of the excited state of each fluorophore (Fries et al., 1998).

$$\tau = \frac{1}{\Gamma + k_{nr}} \quad \text{Eq. [4.1]}$$

$$\tau_n = 1/\Gamma \quad \text{Eq. [4.2]}$$

G. Fluorescence spectroscopy

It is a precise light emission technique in which a light source induces the substance molecules (DaCosta et al., 2003). Then, the relaxation of these molecules occurs by emitting radiation that can be detected, and the intensity is measured (Naresh, 2014). The fluorescence emission spectra represent the vibrational levels in the electronic ground and the excited states respectively (Yamanouchi et al., 1990).

I. Fluorescence spectroscopy components

1. Light source

Several light sources may induce fluorescence emission, such as lasers, light-emitting diodes, arc lamps (which include deuterium, xenon, or mercury), and tungsten-halogen bulbs (Khan et al., 2019).

2. Filters and monochromators

A monochromator or spectrum filter is employed to specify the wavelength of excitation for broad-spectrum sources of light (Brydegaard et al., 2011).

3. Detectors

A photomultiplier tube (PMT), avalanche photodiodes (APD), or a charge-coupled device (CCD) are commonly used to reveal the fluorescence radiation and measure its intensity (Pawley, 2019). Most fluorescence spectroscopy devices employ a photomultiplier tube (PMT) because of its sensitivity to reveal the fluorescence emission at low intensities (Simões & Dong, 2018). It is composed of a vacuum glass tube involving a photocathode where incident light stimulates electron generation, an anode, and many dynos for multiplying electrons (Polyakov, 2013). The photomultiplier's spectral range is influenced by the substance type of photocathode (Seib & Aukerman, 1973). When the

excitation source employed in spectroscopic devices is a laser they are known as laser-induced fluorescence spectroscopy (Hausmann et al., 2014).

II. Laser characteristics in LIF spectroscopy

With lasers, as opposed to broad-spectrum sources, it is possible to excite fluorophores very specifically because they are monochromatic, extremely coherent, and highly directional (Shin et al., 2021). Any wavelength, ranging from the near ultraviolet to the near-infrared, can be available using laser devices (Johansson & Pettersson, 1997). Spectroscopic devices supplied with lasers may significantly minimize or even eliminate interference. Furthermore, real-time acquisition of high sensitivity, high temporal and spatial resolution, and concurrent detection of several factors (Rajapaksha et al., 2019). Pulsed lasers are the most prevalent type used in LIF spectroscopy (Daily, 1997). Their higher power makes them distinct from continuous lasers (Petrash, 1972). High peak power pulses enable frequency mixing, doubling, or tripling in non-linear material, such as crystals (Lin, 1990). This results in the generation of high-powered UV and VUV laser pulses, which enable LIF to be applicable in many fields (Raarup, 2001).

III. Tunable femtosecond laser in LIF spectroscopy

Femtosecond laser sources are commonly employed due to their superior characteristics over nanosecond and continuous laser sources, including a shortened pulse duration and a more substantial peak intensity. Additionally, laser pulses can be centered in a small area, resulting in a highly significant power density (Hannaford, 2004). Concerning living organisms, the main significant characteristic of femtosecond laser systems is their avoidance of heat influence due to the short exposition time through the fs pulse, which has a favorable impact on the survival of biological samples (Vyunisheva et al., 2023). The femtosecond laser's electric field is either equivalent to or superior to the nucleus's Coulomb field, making atomic-level detection feasible (Krainov & Smirnov, 2002). Laser-based approaches to diagnosis were substantially enhanced with the advent of femtosecond (fs) lasers (Xie et al., 2021). In LIF, the femtosecond lasers have several benefits, including being adaptable Femtosecond lasers in LIF offer numerous advantages over conventional

spectrofluorometers, such as being tunable sources with higher specificity and sensitivity that enable LIF to be employed for a variety of applications (Valeur & Berberan-Santos, 2013). The Ti: sapphire laser device is the most predominantly employed femtosecond laser source in spectroscopic techniques (Fisher et al., 1997).

H. The working principle of LIF

Laser-induced fluorescence spectroscopy (LIF) works based on exposing the sample under study to accurate, monochromatic laser light to induce a fluorescent response (Ghervase et al., 2010). The key principle of LIF spectroscopy in the detection of microorganisms is that diverse microorganisms, such as bacteria, viruses, fungi, etc. differ in their molecular structure and have a variety of fluorophores, including coenzymes and amino acids (Hill et al., 2013). When they are stimulated by laser radiation at a specific wavelength, distinct fluorescence emission spectra are obtained (Ramanujam, 2000). Consequently, fluorescence emission spectra are considered as a fingerprint and the fluorescence intensity is highly proportional to the concentration (Pu, 2017; Sierra et al., 2005). These characteristics allow laser-induced fluorescence (LIF) spectroscopy to be an efficient approach for the identification and classification of microorganisms like pathogens and facilitate the creation of an exact device for real-time detection (Hülseweh & Marschall, 2013).

I. LIF spectroscopy advantages

It is a fast detection method (Utkin et al., 2011), requiring little to no sample methodology (Lymer et al., 2020), and technically easy (Ahmed et al., 1978). When applied in optimal circumstances, LIF is a non-invasive, non-contact detection method that maintains the objective surface intact (Fotakis et al., 2006). Technological developments, particularly in spectroscopic instruments, enable LIF devices to be portable and facilitate real-time and outdoor detection (Angheluta et al., 2008; Marques da Silva & Borissovitch Utkin, 2018). Compared to near-IR (infrared) spectroscopy, LIF is a non-destructive technique

that can be sensitive at long distances (Gameiro et al., 2016; Minasny et al., 2009). Employing the UV laser as the excitation source has been demonstrated to significantly increase LIF spectroscopy sensitivity (Sivaprakasam et al., 2004).

J. LIF spectroscopy applications

This method has evolved from its origin in the 1970s into an analysis approach in a wide variety of applications, including industry, biochemistry, biophysics, medicine, ecological preservation, and even heritage studies (Borisova et al., 2013; Grönlund et al., 2006; Spizzichino et al., 2015; Utkin et al., 2014). Based on intrinsic fluorophores in bacteria, laser-induced fluorescence (LIF) spectroscopy is particularly efficient for identifying bacteria (Du et al., 2022a).The LIF technique was used to differentiate between *E. coli* and *E. faecalis* by irradiating them with femtosecond laser pulses (100 fs) delivered by the INSPIRE HF100 laser system which was pumped by a mode-locked femtosecond Ti: sapphire MAI TAI HP laser, the repetition rate was 80 MHz. By analyzing the LIF spectrum of the two bacteria, it was found that the fluorescence emission spectrum of *E. coli* represents a fluorescence peak at 502 nm, and the fluorescence peak of *E. faecalis* was at 512.5 nm. *E. coli* and *E. faecalis* have different LIF fluorescence peak central wavelengths and FWHM, which might be used as a fingerprint (Ezzat et al., 2024).

2. Conclusion

This article review discussed the most prevalent techniques employed for bacterial identification, the microbiological culturing method, enzyme-linked immunosorbent assay (ELISA), and PCR techniques. It was found that the highly costly chemicals used, the long time to obtain results, and the complicated operation methodology constitute significant drawbacks of these techniques. Thus, this review pointed out the femtosecond LIF spectroscopy technique that is highly recommended as a rapid technique for bacterial detection throughout

numerous samples because it is an easy, reliable, and affordable way to identify pathogens without the need for labelling.

Funding: This research received no external funding.

Data availability: No datasets were generated or analyzed during the current study.

Conflict of interest: the authors declare no conflict of interest.

Competing interests The authors declare no competing interests.

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