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# DNA recovery from environmental samples on suspension columns under a combined action of ultrasound and magnetic fields followed by polymerase chain reaction detection

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**A new method is proposed for dynamic DNA extraction from environmental samples (soil, water) on suspension columns containing silica-coated magnetic sorbents ($Fe_3O_4@SiO_2$) under a combined influence of ultrasound and magnetic fields. The recovered DNA was subsequently determined by real-time polymerase chain reaction on an ANK-32 device. It has been shown for the first time that the suspension columns enable the preconcentration of DNA (taking *Mycobacterium tuberculosis* as an example) in a dynamic mode from large volumes of water and soil extracts (100 ml and more) to be performed.**


Pathogenic microorganisms and viruses can occur in environmental samples (natural water, soil, *etc*.) and in wastewaters. The identification of DNA as a bioindicator of causative agents of diseases in such samples can be difficult because of their low concentrations in water and aqueous solutions and also due to substance inhomogeneity materials like soils. Note that environmental samples can contain high-virulent microorganisms that make their timely detection to be important in the control of epidemiologic situation. Thus, the effective recovery and enrichment of DNA as a bioindicator of causative agents of diseases and their subsequent qualitative and quantitative detection is an important problem to be solved. The recovery efficiency (recovery rate) is a ratio of the extracted DNA content to the total DNA content of the initial sample (usually in percent).

In the analysis of soil samples, the determination of DNA is complicated with high amounts of organic substances, in particular, humic substances (HSs) which are inhibitors of polymerase chain reaction used for the specific amplification and detection of DNA.[1]

Almost all sorption and extraction methods for DNA recovery are time-consuming batch and multistage procedures.[2,3] Thus, more than 1 h (15 stages) is needed for the recovery of DNA from soil samples.[2] Most of frequently used methods for DNA recovery from aqueous soil extracts do not make it possible to clean up the samples from interfering substances, such as HSs which complicate the further identification of genetic material.

Ultrasound can have a great effect on the intensification of sorption and desorption processes for different sorbates, including nucleic acids.[4–7] As far as nucleic acids are concerned, any methods for their detection in large volume samples (>100 ml) are not known.

For the recovery and enrichment of DNA from relatively large volumes, it was reasonable to utilize a device, which enables the processes in an automated mode to be performed. We used a flow device with a 1-ml suspension column. The device provides an ultrasound field with a frequency of 3 MHz and an energy density of 6 J $m^{-3}$, the thermostatting of the working column volume, and the consecutive pumping of rinsing and eluting solutions with a rate of 1 ml $min^{-1}$ by a peristaltic pump (Figure 1).

The column contained a silica-coated sorbent with beads of average size 5 μm bearing, however, a significant amount of particles smaller than 5 μm. Such small particles are not completely retained in an aqueous flow by the ultrasound field. For the quantitative retention of the sorbent, including its small-grain fractions, it was necessary to apply a supplemental ‘retaining’ field created by permanent magnets. Correspondingly, magnetic sorbents were needed.

In this work, we used suspension columns with a silica-coated magnetic sorbent ($Fe_3O_4@SiO_2$) to recover DNA in combined acoustic and magnetic fields. The magnetic sorbent was produced by a novel two-step procedure. $Fe_3O_4$ nanoparticles were synthesized using a modified co-precipitation technique,[8] followed by the formation of silica shell onto $Fe_3O_4$ core using the Stöber method.[9] The obtained magnetic sorbent was characterized by transmission electron microscopy (JEOL JEM-200) and static light scattering (Shimadzu SALD-7500nano, Japan).

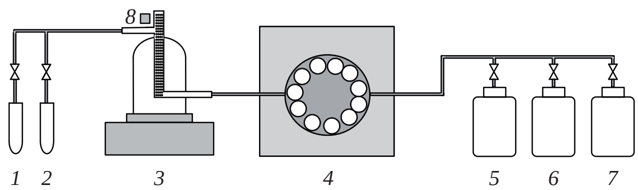


**Figure 1** Schematic diagram of the flow system with a sorbent suspension column: (*1*, *2*) collectors for eluate and washing solution, respectively, (*3*) suspension column, (*4*) peristaltic pump, (*5*) sample, (*6*) washing solution, (*7*) eluent, and (*8*) permanent magnet.

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The application of ultrasound suspension columns allows one to intensify the sorption process due to acoustic microflows, which increase mass transfer in the liquid phase.[10] Simultaneously, the ultrasound field in the column retains the sorbent as a suspension held in a fluid flow. The permanent magnets (10 × 10 × 4 mm, NdFeB) in the upper part of the column (height, 20 mm; inner diameter, 5 mm) served as magnetic filters. This made it possible to use a sorbent mass of about 20 mg completely retained in the system even when the acoustic field alone could not retain smaller sorbent fractions in a sample flow. Using this device, we provided the highly efficient recovery of DNA from water and aqueous soil extracts and obtained ultimate volumes suitable for the subsequent PCR analysis.

Plasmid *M. tuberculosis* DNA was experimentally recovered from real water samples (tap water) and aqueous soil extracts. The test samples were prepared by adding a certain amount of DNA (1000 copies $ml^{-1}$) to the water and soil extracts. To obtain soil extracts, a soil sample (250 mg) containing DNA was suspended in 10 ml of distilled water for 10 min. Then, the solid residue was separated from the solution, which was analyzed. Before the detection, the water or soil extracts were treated with a lysis solution (a mixture of the chaotropic agent guanidine isothiocyanate, EDTA and Tris-HCl). The resulting solution was introduced into the suspension column at a rate of 1 ml $min^{-1}$. The consecutive processes of DNA sorption on the magnetic sorbent (pH 7.5), sorbent rinsing, and DNA desorption (pH 8.0) were performed using ethanol as rinsing solution and thioglycerol as eluting solution ($V$ = 1 ml, a rate of 0.5 ml $min^{-1}$). A 10-μl portion of DNA eluate was taken for the detection by real-time polymerase chain reaction (RT-PCR) on an ANK-32[†] set-up using calibration samples.

The results of batch and dynamic recoveries of DNA from tap water samples were compared (Table 1). The proposed dynamic version of DNA recovery on a suspension column made it possible to enrich up to 80% of DNA. The conventional batch version of DNA recovery resulted in the extraction of only 3% DNA. The batch mode included the lysis of a 200-μl sample followed by adsorption on the silica sorbent and subsequent elution.

The aqueous soil extracts were obtained from soil samples of sod-podzolic sandy loam (SPS) and typical black earth loam (TBE). The recovery of DNA from SPS was as much as 80%, whereas the extraction from TBE reached 60%, which was sufficient for DNA detection. Different recoveries of DNA from the soil extracts can be due to different organic matter contents of soils; in the case of TBE, this could result in a lower extraction rate (the organic matter contents of SPS and TBE are 2.01 and 7.77%, respectively).[‡]

**Table 1** The recoveries of DNA *M. tuberculosis* from test samples ($n$ = 4, $P$ = 0.95).

| Sample | Method | DNA recovery (%) |
|---|---|---|
| Tap water | Batch mode | 3 ± 2 |
| Tap water | Dynamic mode on a suspension column | 79 ± 15 |
| Soil extract (sod-podzolic sandy loam) | Dynamic mode on a suspension column | 80 ± 11 |
| Soil extract (typical black earth loam) | Dynamic mode on a suspension column | 60 ± 15 |

Thus, an effective flow method was proposed for the recovery of DNA from water and aqueous extracts based on sorption extraction and preconcentration with the subsequent elution and detection by RT-PCR.

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[†] The thermal cycler for PCR was fabricated at the Institute of Analytical Instrumentation (St. Petersburg, Russian Federation).

[‡] Certificate of Analysis. Sod-podzolic sandy loam and typical black earth loam (Institute of Agricultural Chemistry, Moscow, Russian Federation).