APPARATUS FOR THE AUTOMATIC DETERMINATION OF OXYGEN CONSUMPTION IN FISH

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ABSTRACT

An apparatus is described which permits the automatic determination of the oxygen consumption of three fish and a control for 24 hours per day. This is made possible by an electrical control system operating four three-way valves which allow water from one of four respiration chambers at a time to flow past an oxygen electrode for quarter-hour periods. These values are registered successively on a recorder.

INTRODUCTION

Oxygen consumption of animals is often taken as a general measure of the intensity of metabolism – the assumption being that all energy is released aerobically. This is commonly measured by one of three methods (Fry 1964; Beamish & Dickie 1967). These are:

i. Measuring the depletion of oxygen in a sealed container (closed system);
ii. measuring the loss of oxygen and the rate of flow of water through a small chamber;
iii. manometric methods.

The first two methods are frequently applied to fish of different sizes. In the first, closed, system the investigator is faced with the difficulty of maintaining constant conditions, since oxygen decreases and carbon dioxide increases during the experiment. Because the concentrations of these two gases affect respiration (Beamish 1964 *a*, *b*; Fry 1964. 1971; Beamish & Dickie 1967) the respiration chamber should be flushed at regular intervals.

The second, continuous flow, system has the obvious advantage that conditions remain constant during the course of the experiment. Since oxygen consumption is calculated from the difference in oxygen content between the inflow and the outflow (together with the rate of flow) a high degree of accuracy is, however, necessary in the determination of oxygen content and flow-rate.

Winkler titration, which has been used since the earliest oxygen consumption studies to determine the oxygen content of aqueous solutions, is a very reliable though tedious method. More popular and extremely convenient, are the various designs of oxygen electrodes which continuously monitor the oxygen concentration of water leaving the respirometer. These electrodes, which are normally calibrated in oxygen-free and in air-saturated water from time to time, have been used extensively in oxygen determination studies in recent years. Normally one

fish at a time can be monitored by this method, or otherwise the probe has to be manually rotated between as many respirometers as are in use.

In this paper an apparatus is described which automatically performs this task.

APPARATUS

General

The general outlay of the system is presented in Figure 1. Water to the respiration chambers is supplied by container A and the flow is maintained by gravity. Polythene tubing with inside diameter 7 mm and outside diameter 11 mm was used for all connections. The thickness of the tubes (2 mm) ensures minimal heat loss and gas diffusion. To overcome the problem of bacterial and algal growth inside the polythene tubes, two complete sets of tubes were used. After being used for a day, one set was removed and replaced by the other set which had been cleaned and sun-cured for at least five hours.

The outlet tubes of the respiration chambers (B1–B4) are connected to four valve systems (C1–C4) which allow water from one respiration chamber at a time to flow past the oxygen probe (D).

The probe and flow-through cell as well as the four respiration chambers are submerged in water-bath E. The temperature of both this bath and that of the holding tank is regulated by thermo-regulators F1 and F2 to within 0,1°C.

To match impedance a parallel resistor (G) was placed between the oxygen metre (H) and the recorder (I).

Water flow through the respiration chambers is regulated and equalized by adjustable supports (J). This is necessary since rate of flow affects oxygen meter recordings and because flow-rate past the probe and through the by-passes must be equal. This is achieved by setting the adjustable support of any respiration chamber at the desired level to obtain a specific flow-rate

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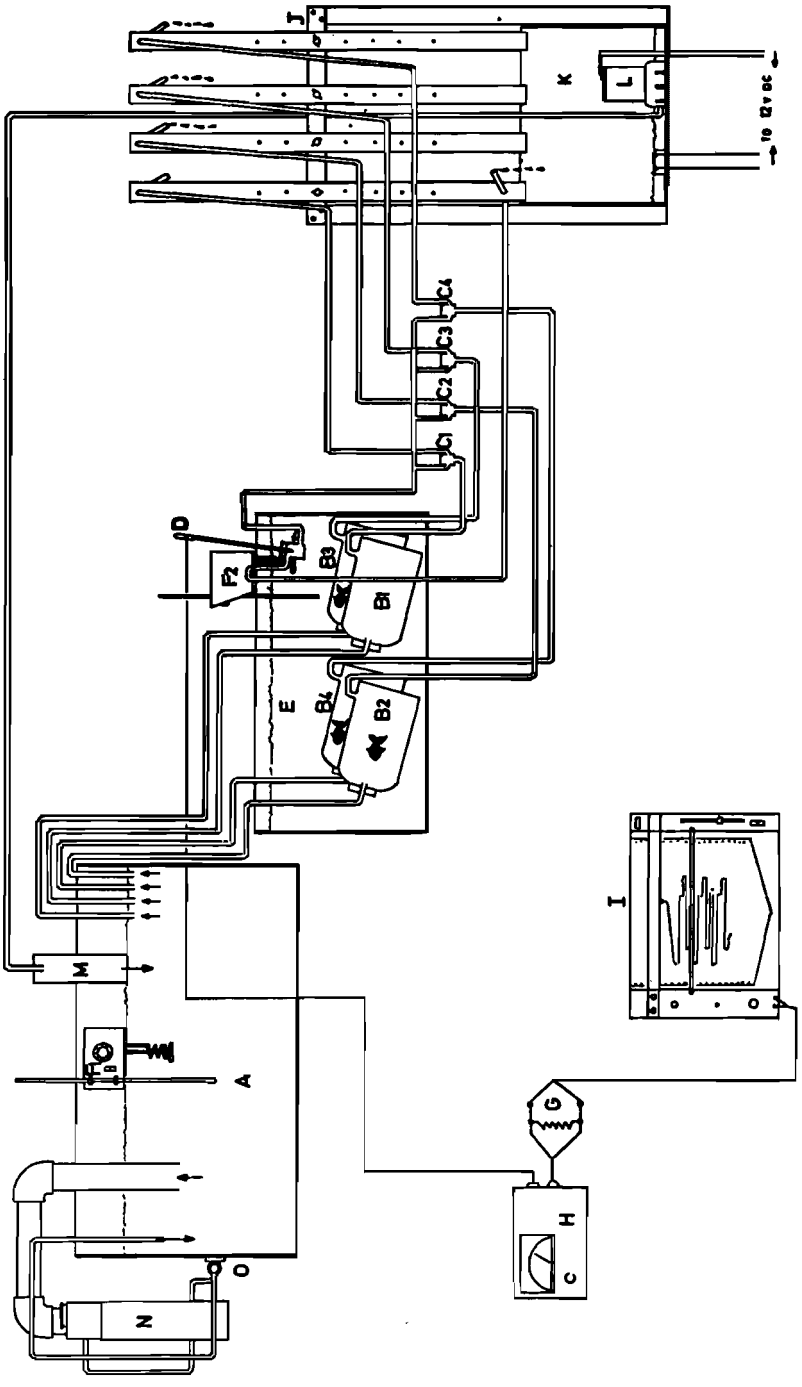
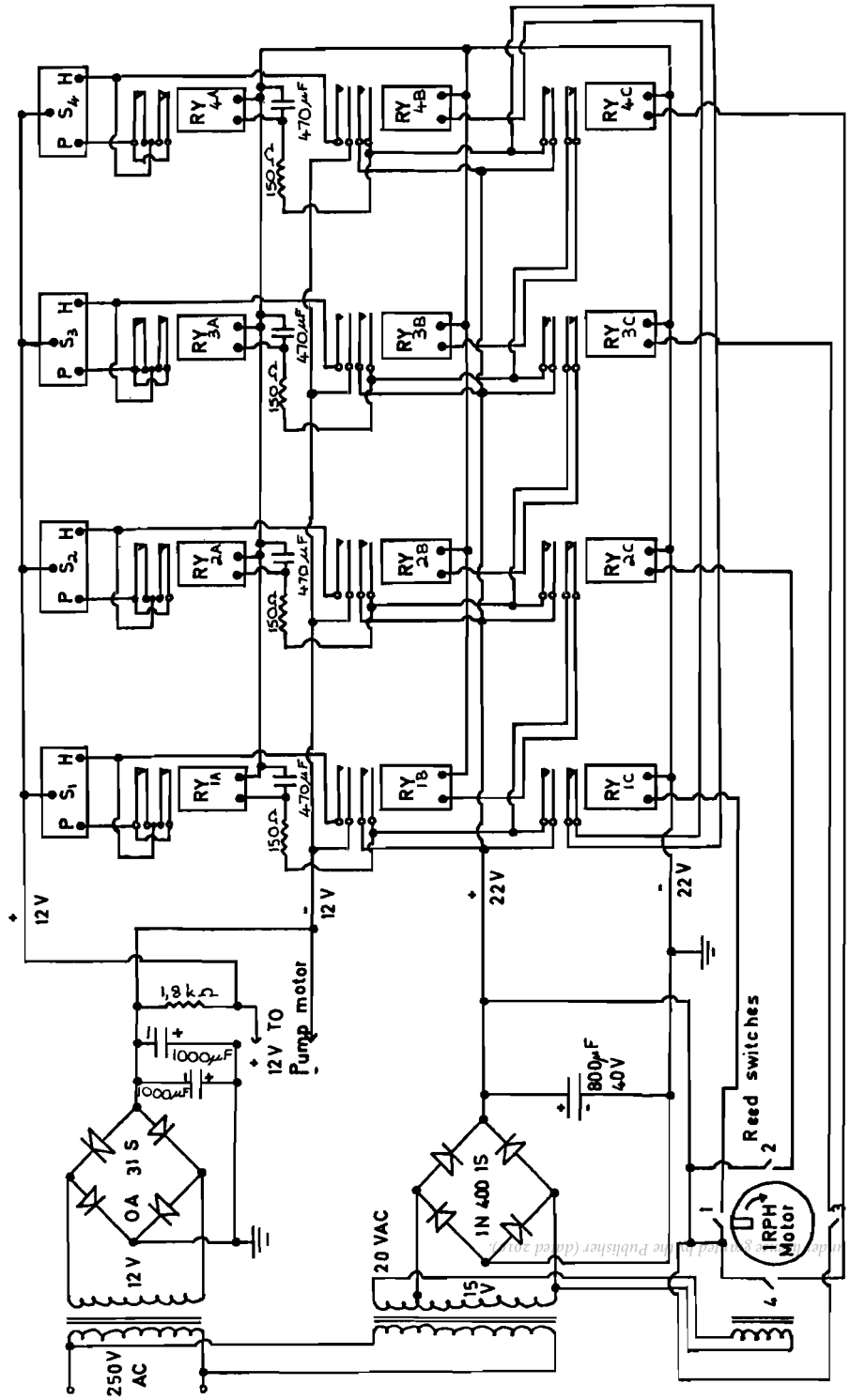
FIGURE 1

General outlay of apparatus for determining oxygen consumption.

A. 500 litre PVC water tank.
B1–B4. Four ten-litre aspirator Pyrex bottles.
C1–C4. Four valve systems (presented in Figure 3).
D. Oxygen probe.
E. Glass container functioning as water-bath.
F1 & F2. Thermoregulators (Thermomix 1460 B. Braun Melsungen AG).
G. Parallel resistor.
H. Oxygen meter (Oxi 39, Wissenschaftliche – Technische Werkstatten, D 812 Weilheim).
I. Recorder (Servograph Pen Drive R.E.A. 310 equipped with R.E.A. 112 high sensitivity unit).
J. Adjustable supports to regulate flow rates.
K. Glass tank.
L. Pump (Bilge King Pump, Crowell).
M. Filter containing glass wool.
N. Protein skimmer (Tunze Turbella 4 000).
O. Ultra-violet tube (Philips).





past the probe and subsequently setting the flow-rates of the four by-passes. Flow-rates are determined by making use of an accurately calibrated measuring cylinder.

Outflowing water is collected by a glass container (K) and pumped back by means of a bilge pump (L) to container A. A floating mercury switch effects this at regular intervals of $\pm$ 8 minutes (which corresponds to $\pm$ 12 ℓ of water). This ensures that water level and thus flow-rate (which was measured twice daily and never fluctuated by more than 1 per cent) remains constant.

The end of the delivery pipe is fixed into a glass wool filter (M). Water is oxygenated while dropping from the outlets and by air stones in container A.

The water in container A is continuously circulated through a filter and protein skimmer (N) as well as being sterilized by means of ultra-violet light (O). The UV source is a modified 0,6 metre-long Philips tube to which is added an outer sleeve through which a thin layer of water can flow. At the achieved flow-rate of 5 litres per minute all the water in the system is recycled at least 14 times per day.

Electrical control system

A circuit diagram of the electrical system operating the valves is presented in Figure 2.

The system was devised to operate the four valve systems to alternately divert the water flow through the flow-through cell containing the oxygen probe for quarter-hour periods. As suitable valves were unobtainable locally, GEC automatic washing-machine valves, which are constructed out of acceptable materials, were modified and used. These valves can be classified as three-way valves (*i.e.* one inlet and two outlets) which divert the flow of water either past the probe or to the by-passes.

The solenoid coils fitted to these valves were found to be inadequate and were replaced by stronger ones. These coils were designed so as to have separate pull-in and hold-in windings to minimize heat production as water temperature must remain constant. The hold-in power is considerably less than the pull-in power. The circuit diagram (Figure 2) shows a one-revolution-per-hour motor, which is a synchronous motor geared down to give accurate time periods. The motor drives a disc with a magnet which operates reed switches which are used for reliability.

Operation is as follows: reed switch '1' closes, causing relay RY_{1C} to operate and supply power to RY_{1B}. When RY_{1B} operates, it closes a hold-in contact which will keep RY_{1B} pulled in after RY_{1C} releases due to the reed switch '1' opening. RY_{1B} also supplies power directly to the hold-in contact of solenoid 'S_1' and RY_{1A}, and indirectly, through the contact RY_{1A} to the pull-in winding on 'S_1'. Due to the time delay network consisting of the 150 ohm resistor and 470 microfarad capacitor in the supply to RY_{1A}, this relay only operates after about a quarter of a second, cutting the supply to the pull-in winding of solenoid 'S_1'; thus the solenoid only has power supplied to the pull-in winding long enough to operate it. As the magnet rotates, reed switch '1' opens, RY_{1C} releases, but RY_{1B} and RY_{1A} remain operational due to the hold-in

FIGURE 2

Circuit diagram of the electrical system operating the valves and bilge pump.

contact on RY_{1B}. When reeds switch '2' operates, the same sequence takes place with RY_{2C}, RY_{2B}, RY_{2A} and 'S_2'. In addition, RY_{2C} opens the hold-in circuit for RY_{1B} so that as 'S_2' operates, 'S_1' is released. This sequence continues for 'S_3', S_4, S_1, etc. and thus precludes the possibility of more than one solenoid being held in at any time.

The 12 volt DC supply also powers the pump motor which is controlled by a water-level operated switch.

Operation of valves

A diagrammatic illustration of one of the valves is presented in Figure 3. The valve is normally open to port B1 – *i.e.* water flows from A via diaphragm valve D1 to B1. The illustration (Figure 3) shows the solenoid in the energized state. In this state the coil pulls steel core J in the direction of the arrow X, thus turning the actuating lever H on fulcrum I in a clockwise direction.

This lever then shuts down port B1 via plunger G1 that seals the diaphragm valve on the seat of nylon body C. At the same time pressure is released on plunger G and the water pressure moves diaphragm D upwards, thus allowing the water to flow from inlet port A to outlet port B as shown by the arrow. When the coil is de-energized, spring L returns the mild steel core and actuating lever to the normal position, thus closing port B and opening port B1. The thin rubber diaphragm E is essential to avoid water leakage through plunger retainers F.

RESULTS

With this system the individual oxygen consumption of more than 100 mullet varying in size from two to more than 200 g has been successfully determined at various conditions of temperature and salinity. Of these some are presented in Figure 4. Although recordings start immediately after the introduction of fish into the containers, measurements are taken after not less than eight hours. This precaution is taken to ensure that fish are calm and respiring normally. The mean of at least five night- and five day-determinations is taken when calculating the daily oxygen consumption of a fish.

Twenty-four hours after renewing the oxygen electrode system and calibrating the probe, the probe is rechecked and reset if 'drift' has taken place. Subsequent checks are performed every two days although recalibration is seldom necessary. The electrode system is renewed after seven days and the same procedure repeated.

The accuracy of oxygen determinations is determined by the accuracy of the apparatus. This is given by the manufacturers as $\pm$ 1 per cent of full scale at the calibration temperature; $\pm$ 3 per cent of full scale at 5°C deviation of calibration temperature from measuring temperature; $\pm$ 5 per cent of full scale at 10°C deviation of calibration temperature from measuring temperature.

One of the main advantages of this system is that minor fluctuations in temperature, and thus in oxygen content of the water, are automatically taken account of. This is demonstrated in Figure 4, A and C. In Figure 4A all four respiration chambers experience the same drop in oxygen content due to a slight increase in temperature. In Figure 4C the opposite effect is demon-

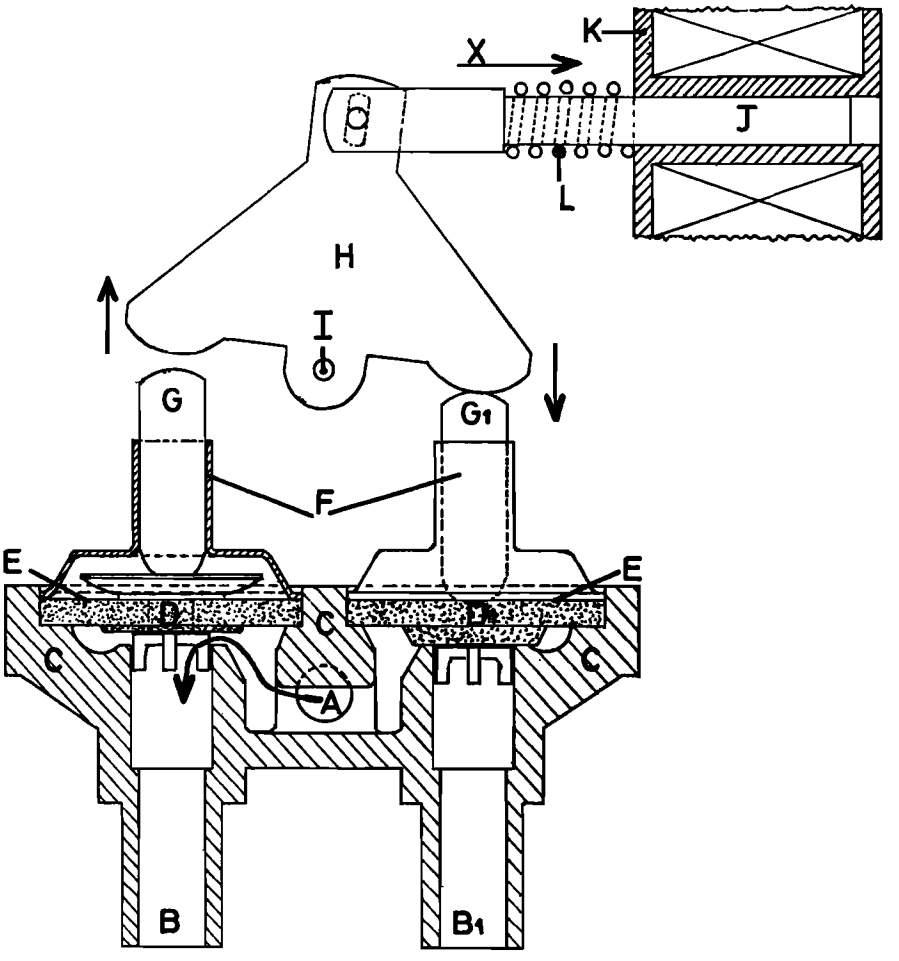
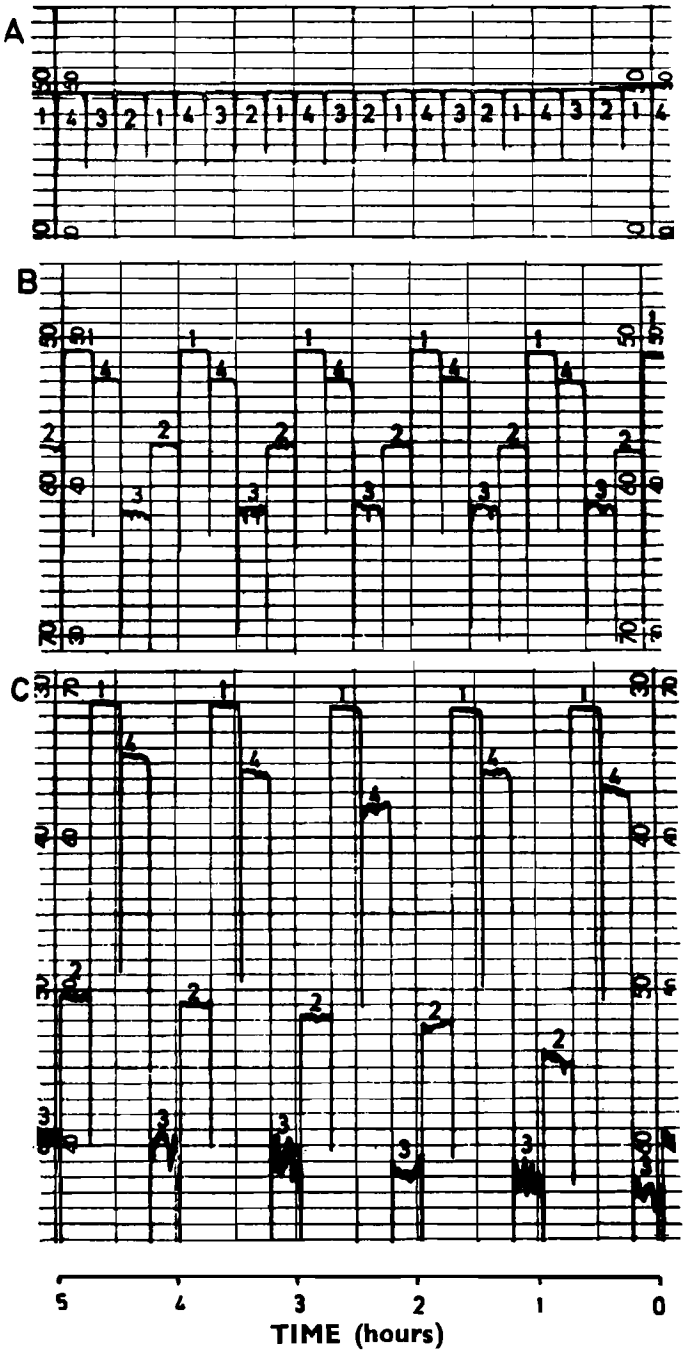


FIGURE 3

Illustration of one of four identical valves used to divert water flow to the oxygen electrode for quarter-hour periods (C1–C4 in Figure 1).

A. Inlet port.
B & B1. Outlet ports.
C. Nylon valve body.
D & D1. Neoprene diaphragm valves.
E. Thin rubber diaphragm.
F. Plunger retainers
G & G1. Nylon plungers.
H. Actuating lever.
I. Fulcrum.
J. Mild steel core.
K. Solenoid.
L. Spring (compression).



strated. This, however, does not affect the accuracy of oxygen consumption values since all four chambers experience the same drop or increase in oxygen content. Further, the actual consumption by any fish is measured as the difference between the control and the outflow of any fish respiration chamber. The fluctuations observed in Figure 4C are true reflections of fluctuations in the oxygen consumption of the fish.

To obtain the actual amount of oxygen used during any quarter of an hour period, the number of divisions separating the control line and the consumption line of any fish is multiplied by the flow-rate and the oxygen value represented by one division. (The deflection of the recorder was calibrated with that of the oxygen meter.)

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FIGURE 4

A. The oxygen content of water flowing through four respiration chambers without any fish.

B. Oxygen content of water from respiration chambers containing:
(1) control; (2), (3) and (4) fish weighing 10,9; 12,1 and 5 g respectively.

C. Oxygen content of water from respiration chambers containing:
(1) control; (2), (3) and (4) fish weighing 58,5; 92,5 and 5,2 g respectively.

The water flow-rate in these examples was 380 ml/min.